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SUMMARY

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REACTION OF PLUM CULTIVARS AND ROOTSTOCKS TO BACTERIAL BLIGHT (*PSEUDOMONAS* SP.)

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Abstract

The bacterial blight caused by the Gram-negative bacteria Pseudomonas sp. is a significant problem in stone fruit orchards. The resistance or tolerance of the cultivars is one important strategy for disease control. In the frame of a study conducted with the support of the BNSF, administrative contract КП-06 М 46/2, was compared the reaction of plum cultivars and rootstocks, after inoculation with the bacterial pathogen Pseudomonas sp. Flowers and shoots of the cultivars 'Topgigant Plus' and 'Jojo' grafted on the two rootstocks - 'Docera 6' (Prunus domestica L. x Prunus cerasifera Ehrh.) and the seedling myrobalan plum rootstock (Prunus cerasifera Ehrh) were artificially inoculated. In addition, two years old rootstocks 'Docera 6', seedling P. cerasifera and 'Myrobalan 29C' (Prunus cerasifera) also were inoculated with bacterial suspension. The cultivar 'Topgigant Plus' had lower susceptibility to flower infection when grafted on the seedling rootstock than 'Docera 6'. The reaction of rootstocks fourteen days after inoculation showed a lesion diameter of 400 mm on 'Docera 6', 433 mm measured on 'Myrobalan 29C', and 41 mm on the seedling rootstock P. cerasifera.

Key words: *Artificial inoculation, 'Docera 6', 'Jojo', 'Myrobalan 29C', Pseudomonas sp., Topgigant Plus.*

INTRODUCTION

The genus *Prunus* contains over 400 species, including the European plum. *Prunus domestica* L. is a traditional fruit crop in Bulgaria (Bozhkova & Savov, 2016). The South Central Region represents 22.2% of the total area occupied by plum trees and this culture occupies third place after walnuts and sweet cherries concerning planted areas (Ministry of Agriculture and Food, 2021). Bacterial canker, caused by members of the *Pseudomonas syringae* species complex, can be a major limiting factor in the cultivation of *Prunus* spp. (Omrani et al., 2019; Vicente et al., 2004). The diseases of fruit trees caused by the strains of *P. syringae* are resulting in severe economic losses (Gomila et al., 2017; Lee et al., 2015; Young, 2010). Due to a lack of effective control measures, plant diseases caused by bacteria are a significant problem for the global horticultural industry (Sundin et al., 2016; Lee et al., 2023). Independently of the genotype used as rootstock, the most important cultural practice for avoiding tree losses caused by *Pseudomonas* and other wound parasites is to avoid damage to the stem (Hinrichs-Berger, 2004). The disease caused by bacteria of *Pseudomonas* sp. is primarily characterized by necrosis, gummosis and dieback of woody plant tissues. In addition, the pathogens colonize other plant tissues where they exist epiphytically or invade to cause leaf and fruit spots and blossom blight. These tissues can be reservoirs for later woody tissue infection (Crosse, 1966). The phytopathogenic bacteria *P. syringae* is associated with several plant species (Gašić et al., 2018; Kennelly et al., 2007; Lee et al., 2015; Ruinelli et al., 2019), including fruits and ornamental plants (Scortichini et al., 2003; Vicente and Roberts, 2007). It is a prevalent bacterial pathogen that can incite stem and leaf diseases in various crop plants, particularly in temperate regions (Scholz-Schroeder et al., 2001). *P. syringae* complex infects woody tissue, exhibits the symptoms of cankers, and eventually spreads to the entire wood and kills branches (Gomila et al., 2017; Perminow et al., 2018).

The rapid laboratory-based tests allowing screening for tree resistance is a major challenge underpinning the rapid development of new cultivars that resist pests and diseases (Hulin et al., 2018). Bacterial canker

(*Pseudomonas syringae* van Hall) is an important disease in most plum-producing countries. According to Ramming and Cociu (1991), plums grafted on peach rootstock are less susceptible than those on plum rootstock. 'Myrobalan' rootstock is less susceptible than 'Marianna' rootstocks. Nothing is known about the inheritance of resistance to bacterial canker in plums. It remains essential for future breeding work to find sources of resistance against bacterial canker. A resistance test was developed for sweet cherries, which could be adapted to plums (Santi et al., 2004).

The rootstock Marianna (a hybrid of *P. cerasifera* and a native plum), is reported as prone to infection (Wilson et al., 1953). Seedling myrobalan plum (*Prunus cerasifera*) is much more resistant as are most mazzard seedlings. The Myrobalan B, Purple Pershore and Mahaleb were supposed resistant. Grubb (1937) does not feel that there is any direct evidence that rootstocks affect the resistance of the scion. If such an effect does exist, it is thought to be due to the influence on vigor.

Differences in susceptibility to different parts of the disease cycle of *Ps. syringae* have been reported on many occasions. Growers have noted that certain cultivars in mixed plantings were more tolerant of infection than others. Lists have been published by many research centers giving the degree of susceptibility of the cultivars in their experimental plantings. These lists have varied widely depending on the area, the phase of the disease being considered, and the horticultural practices. The degree of resistance, or tolerance, of a particular cultivar, frequently depends on its stage of development concerning the time that inoculum is available. Because of differences in the time of infection and climatic conditions, cultivars listed as resistant in one part of the world are classified as very susceptible in another region. Within the *Prunus* species, apricot is listed as the most susceptible, sweet cherry and some plum cultivars as second, followed by nectarine (Barss, 1915; Cameron, 1962).

Our study aimed to compare the reaction of different tree parts of plum cultivars and rootstocks after artificial inoculation with the bacterial pathogen of *Pseudomonas* sp.

MATERIALS AND METHODS

Disease symptoms of bacterial blight were observed and the phytopathogen was collected from an experimental plum orchard at the Fruit Growing Institute-Plovdiv, Bulgaria. In the growing seasons of 2021 and 2022, a survey has been conducted to investigate the symptoms of plum canker, lesions on branches, twigs and shoots as well as angular spots on the leaves and blight on flowers. The symptomatic tissues from flowers, shoots and branches cut were into squares using a scalpel and the surface was disinfected with 75% ethyl alcohol. The samples were washed 3 times with distilled and sterile water, and tissues were placed on King's B media. A single colony of potential strain was transferred on King's B media after 48 h conducted for fluorescent pigment. The obtained isolates were infiltrated into a tobacco leaf's mesophyll to detect a tobacco hypersensitivity (HR) reaction. The bacterial suspensions was 10^8 cfu/ml, positive response to white necrosis at the infiltrated area 24 h after inoculation indicates the ability to induce an HR. To assess the pathogenicity of pathogens green plum fruits (cultivar 'Topgigant Plus'), which were surface disinfected were artificially inoculated with bacterial suspensions (10^6 cfu/ml) by injecting. For negative control were used green plum fruits injected with sterile water. All plum fruits were covered with plastic bags.

As a plant material in the experiment were used blossoms and shoots of the cultivars 'Topgigant Plus' and 'Jojo' grafted on the two rootstocks - 'Docera 6' (*Prunus domestica* L. *x Prunus cerasifera* Ehrh.) and the seedling myrobalan plum rootstock (*Prunus cerasifera* Ehrh) which were artificially inoculated.

The study used also two years old plants of the rootstocks 'Docera 6', the seedling *P. cerasifera* and the clonal 'Myrobalan 29C' (*Prunus cerasifera*).

For artificial infection of the blossoms were used 1-year-old shoots, from trees show no symptoms of diseases. A minimum of 200 flowers were used in each repetition for each cultivar/rootstock combination. They were cut in BBCH 57 phenophase and placed in water containers. The inoculation was done by

spraying the opened flowers (BBCH 65) with bacterial suspension (10^6 cfu/ml). Sprayed shoots were covered with moist plastic bags and maintained in favorable conditions (t = 23°C and 80-90% relative humidity) for 6 days. Symptoms development was monitored daily. After 5 days, the degree of attack was determined for each flower using a 5-grade scale:
0 - no symptoms;
1 - symptoms of necrosis on petals;
2 - symptoms of necrosis on pistil and receptacle;
3 - symptoms of necrosis observed on sepals;
4 - symptoms of necrosis observed on all flower parts.
An average degree of attack was calculated. The level of susceptibility of the flowers was formed in basic degree attack using a 6-grade scale:

0- immune (no infection);
1- resistant (single infected flowers);
2- low susceptible (1-10% infected flowers);
3- moderately susceptible (11-25% infected flowers);
4- susceptible (26-50% infected flowers);
5- highly susceptible (over 50%).

Plum shoots from the cultivars ‘Topgigant Plus’ and ‘Jojo’ grafted on ‘Docera 6’ and *P. cerasifera* were cut in the dormant period and placed in water containers. The shoots' surface was disinfected and inoculated with bacterial suspension (10^8 cfu/ml). Ten shoots in repetition were inoculated by sterile needle and injection of 25µl bacterial suspension.
In the dormant period two-year-old plants of the rootstocks ‘Docera 6’, ‘Myrobalan 29 C’ and seedlings *P. cerasifera* were planted in 3 liters plastic containers. The rootstock surface was disinfected and inoculated using the same methodology as the shoots infection. The shoots and rootstocks were incubated at 25°C for 2 weeks. To compare the reaction of the plum cultivars and rootstocks, lesion blight (mm) were measured in 7 and 14 days after inoculation.
Each artificial inoculation was done with 24h bacterial cultures plates on KB media and incubated at 23°C.
The pathogen was reisolated from all symptomatic tissues, to complement Koch’s postulates and was compared to the original isolates.

RESULTS AND DISCUSSIONS

Bacterial blight in the experimental plum orchard in Plovdiv, Bulgaria, was identified by visual observations and confirmed by laboratory tests. A total number of nine isolates were collected from different trees in the orchard. Of these, only four isolates exhibit fluorescence under UV light. All the studied potential *Pseudomonas* spp. were Gram-negative and positive to the tobacco HR test. The tobacco leaves became hypersensitive 24 h after the introduction of the pathogen into the leaves. The positive reaction was observed in 3 days after inoculating green fruit plums with all tested strains.
Crosse and Benmett (1955) observed the damage caused by *Pseudomonas* spp. on plum orchards. The cankers on plums start as small, brown to reddish-brown spots that enlarge as water-soaked streaks. In the spring, the area between the streaks turns brown and the area becomes uniformly brown and moist (Wilson & Hewitt.,1939; Wormald., 1932). Wilson et al. (1939) also describe gum formation as follows: "As a rule, little if any, gum is exuded from the affected tissues but, a watery material may flow from cracks in the bark and cover the limbs. The absence of gum is particularly noticeable in the case of plums." Cracks appear around the margin of the canker as the dead area dries out.
From all isolates, one was selected for artificial infections. The strain number TG/D007 was gram-negative, had positive HR on tobacco, produced fluorescent pigment after 24 h cultivated on KB media and was positive for pathogenicity test on green fruits.
As a control variant was used the cultivar ‘Stanley’ grafted on *P. cerasifera* rootstock.
Disease severity in cultivar ‘Jojo’ grafted on *P. cerasifera* rootstock was 3.49% that was a high value in the experiment (Table 1). This cultivar/rootstock combination was with statistically significant difference compared to the other studied cultivars. For ‘Jojo’ grafted on ‘Docera 6’ was calculated 2.40% severity of the disease. The difference was statistically significant compared to the ‘Jojo’/ *P. cerasifera*. The control cultivar showed 0.67% severity of the disease.

Table 1. Percentage of infected plum flowers

Cultivar/rootstock	Diseases severity (%)	Degree of attack (%)	Level of susceptibility
Jojo/Docera 6	2.40 b	49.17	susceptible
Jojo/*P. cerasifera*	3.49 a	88.0	high
Topgigant Plus/Docera 6	0.56 c	12.70	moderate
Topgigant Plus/*P.cerasifera*	0.32 d	5.41	low
Stanley/*P. cerasifera*	0.67 c	11.52	moderate

*Different letters in the same row/column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

The disease severity for 'Topgigant Plus' cv. was a lower value compared to cultivar 'Jojo', grafted on 'Docera 6'. The difference was statistically significant.

The susceptibility level in the cultivars varies depending on the percentage of infected flowers of each cultivar. The cultivar 'Jojo' grafted on *P. cerasifera* reacted as highly susceptible with 88% infected flowers caused by *Pseudomonas* sp., while the grafted on 'Docera 6' was evaluated as susceptible to the disease - 49.17% infected plum flowers.

The cultivar 'Topgigant Plus' grafted on 'Docera 6' and 'Stanley' were evaluated as moderately susceptible to the bacterial pathogen. For both cultivars, the percentage of the infected blossoms was of low value - 12.70% for 'Topgigant Plus' and 11.52% for 'Stanley'. The percent of infected blossoms was the lowest for 'Topgigant Plus' when grafted on *P. cerasifera* and according to its reaction the cultivar/rootstock combination was evaluated as low susceptible to the *Pseudomonas* sp. phytopathogen. In this experimental stage the influence of the rootstocks on the reaction to flower infection is negligible.

The attack on flowers reported as blossom blighting occurs on plums (Anderson, 1956) but is not usually as severe as on sweet cherries (Wilson et al.,1939). In England, shoot wilt of plum is quite common and more frequently incited by *Ps. syringae* than by *Ps. mors-prunorum* (Crosse, 1954; Wormald, 1928). However, both can cause the appearance of similar symptoms (Wormald., 1931). The death of dormant buds is not usually important in domestic or Japanese plums.

The pathogenic *Pseudomonas* sp. strain was used for artificial inoculation of cut plum one-year-old shoots and their reaction was evaluated by measuring the infected part after 10 and 20 days. Non-significant difference was observed for the reaction observed for 'Topgigant Plus' shoots from the cultivar grafted on 'Docera 6' and grafted on *P. cerasifera*. Twenty days after infection, the measured lesion was longer - 500 mm for 'Topgigant Plus' cv. grafted on both rootstocks. A similar situation was observed for the cultivar 'Jojo', grafted on 'Docera 6'. Ten days after inoculation the infected part of the shoots was 42 mm, compared to the same cultivar grafted on *P. cerasifera* where the lesion was 37 mm.

Table 2. Lesion diameter (mm) of infected plum shoots

Rootstock	Cultivar	Lesion diameter (mm)	
		10 day	20 days
Docera 6	Togigant Plus	42 a	500 a
	Jojo	42 a	450 a
P. cerasifera	Topgigant Plus	45 a	500 a
	Jojo	37 a	475 a
	Stanley	40 a	400 a

*Different letters in the same row/column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

The infected part of shoots 20 days after inoculation showed a 450 mm lesion for the cultivar grafted on 'Docera 6' compared to 475 mm for the cultivar grafted on the seedling rootstock. The control variant 'Stanley' showed the lowest result compared to other cultivar/rootstock combinations in the experiment. On the 10^{th} day after inoculation, the lesion part was 40 mm, and on the 20^{th} day, it was 400 mm. The screening of plum shoots showed non-significant differences between the studied cultivar/rootstock combinations and the control variant, 10 and 20 days after infection.

The plants of the plum rootstocks were inoculated with a pathogenic strain of *Pseudomonas* sp. and their reaction was evaluated by measurement of the lesion 10 and 20 days after inoculation. On the 10^{th} day was calculated an average value from 5 repetitions of each rootstock. The seedling rootstock reacted with a 33 mm lesion. Non-significant difference was observed between the seedling rootstock and 'Docera 6'. The lesion measured for 'Myrobalan 29 C' rootstock was 283 mm which was the highest value in this experiment.

Table 3. Lesions diameter (mm) of infected rootstock

Rootstock	Lesion diameter (mm)	
	10 day	20 day
Docera 6	83 b	400 a
P. cerasifera	33 b	41 b
Myrobalan 29 C	283 a	433 a

*Different letters in the same row/column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

Twenty days after inoculation a significant increase was observed for 'Docera 6' and the measured lesion was 400 mm. The result was similar to 433 mm measured for the rootstock 'Myrobalan 29 C'. The difference observed between *P. cerasifera* and both other rootstocks was statistically significant.

CONCLUSIONS

In this study, we report plum canker, lesions on shoots and blossom blight caused by members of the *Pseudomonas* sp. on plum orchards as responsible for relevant yield losses in Bulgaria. Isolated nine potential bacterial pathogens and one was selected for inoculation. The selected strain identified as *Pseudomonas sp.* was gram-negative and positive to the tobacco HR test. The pathogen was positive reaction 3 days after inoculating green fruit plums.

In our study, the flowers of cultivar 'Jojo' were evaluated as susceptible to *Pseudomonas* sp, while the flowers of 'Topgigant plus' reacted with low susceptibility to the bacterial pathogen. The value reported in shoot infection showed similar results.

The rootstock does not influence flower infection and lesions on shoots. Minimal differences between the cultivars grafted different rootstocks were observed. The reaction after shoots infection also showed similar results. The rootstocks 'Myrobalan 29 C' and 'Docera 6' were evaluated as more susceptible to *Pseudomonas* sp. than the seedling *P. cerasifera* rootstock.

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CANES WILTING WITH COLLAR AND ROOT ROT OF RASPBERRY CAUSED BY *PHYTOPHTHORA PSEUDOCRYPTOGEA* IN BULGARIA

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Abstract

A wide range of herbaceous and woody plant species are known as host plants of Phytophthora pseudocryptogea, a relatively newly described species. Recently P. pseudocryptogea was isolated from raspberry plants in Bulgaria. Diseases plants were found in the 4 to 5 years old variety Ljulin plants on the biological production orchards in Kostenets region. Canes suddenly wilt and turn brown at the onset of warm temperatures. The plants manifested disease symptoms such as collar and root rot. The precise species determination of obtained isolate was done on the basis of the colony and asexual spores morphology and sequence analyses of the ITS region of the nuclear DNA. The pathogenicity of the fungus was tested by detached leaf bioassay on several raspberry cultivars in the laboratory.

Key words*: raspberry, collar and root rot, Phytophthora pseudocryptogea, Bulgaria.*

INTRODUCTION

Phytophthora pseudocryptogea was distinguished from *Phytophthora cryptogea sensu stricto* and designated as a new oomycete species several years ago (Safaiefarahani et al., 2015). Both of plant pathogens possess a wide host range, but recently was found that they differ in their host range. Both of them are pathogenic to the same plant species as some solanaceous (potato, tomato, eggplant), pistachio, spinach, and clover. But other plant species express different responses to inoculation of both pathogens and thus are able to demonstrate the difference among their host range. The cucumber, melon, watermelon, alfalfa, soybean, cabbage, green pepper and rice, are found to be susceptible to *P. pseudocryptogea*, but are not infected by *P. cryptogea* (Delshad et al., 2020). *P. pseudocryptogea* was reported as a part of the *Phytophthora* pathogen complex responsible of declining of some plants as common alder (*Alnus glutinosa*) trees (Seddaiu & Linaldeddu, 2020) and pomegranate (Ghaderi & Habibi, 2021). In a few years the host range of *P. pseudocryptogea* expands to include more plant species.

In this study, an isolate of *P. pseudocryptogea* was obtained from diseased raspberry plants in Bulgaria and characterized.

MATERIALS AND METHODS

Isolation of *P. pseudocryptogea*

The four and five years old wilting raspberry plants of cultivar Ljulin, grown in the biological production orchards demonstrated collar and root rot. An isolate of *P. pseudocryptogea* was obtained by baiting from a soil sample, taken from the root area of diseased plants. The collected soil sample was distributed on the bottom of plastic boxes and covered by a water layer (1 cm depth). *Rhododendron* leaves were placed on the surface of the water (Themann & Werres, 2000). After developing necrotic areas on the leaves and common surface sterilization, the causal agent of the spots wasisolated on water agar and then transferred on a selective for *Phytophthora* species PARNHB medium (carrot agar supplemented with 10 mg Pimaricin, 250 mg Ampicillin, 10 mg Rifampicin, 50 mg Nystatin, 50 mg Hymexazol and 15 mg Benomyl/1,000 ml) at 25ºC. The developed mycelium then was used for DNA extraction and in pathogenicity tests.

Determination of *P. pseudocryptogea* specie

The determination of the exact species of obtained *Phytophthora* isolate was done by DNA sequence analysis. DNA extraction was done from 10-days old mycelia using DNeasy Plant Mini Kit (QIAGEN GmbH), followed by PCR amplification of the ITS region with primers ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') using PuReTaq^{TM} Ready-To-Go^{TM} PCR beads (GE Healthcare Life Sciences), according to the manufacturer's instructions and the following PCR thermal program: 96ºC - 2 min, 35 cycles of 96ºC - 1 min, 55ºC - 1 min, 72ºC - 2 min, and final elongation at 72ºC - 10 min. The purified PCR product was sequenced in GATC Biotech AG (Germany). The obtained ITS sequene was compared with others by performing a BLAST search in the NCBI (National Center for Biotechnology Information) database.

Morphology of colonies and asexual structures

Phytophthora pseudocryptogea isolate was grown on V8 juice agar (16 g agar, 3 g $CaCO_3$, 100 ml Campbell's V8 juice and 900 ml distilled water), for 10 days in the dark at 20°C. Colony morphology patterns were characterized according to Erwin and Ribeiro (1996). The sporangia production was stimulated according to Jung and Burgess (2009), by flooding cultured V8 agar sections of approximately 20 × 15 mm with non-sterile pond water in 90 mm Petri dishes. After 36-48 hours of incubation at ambient conditions, the dimensions and the characteristic features of 50 mature sporangia chosen at random were determined under microscope at ×400 magnification (ZEISS Axio Imager A2) with a digital camera (AxioCam ERs 5S) and a biometric software (AxioVision LE).

Pathogenicity test

The pathogenicity of the isolate was checked by detached leaf assay on several plant species (perennial woody and shrubby plants: Turkey oak (*Quercus cerris*), sweet cherry (*Prunus avium*), linden (*Tilia tomentosa*), blackberry (*Rubus fruticosus*), cherry laurel (*Prunus laurocerasus*), Japanese knotweed (*Fallopia japonica*). Turkey oak and linden are trees widely spread in forest areas in Bulgaria, as well as in urban green areas. Cherry laurel is also broadly grown as a decorative plant in city parks. Sweet cherry is often found in private yards and in orchard gardens. Japanese knotweed is an invasive plant in the country that is easy to be seen in many different habitats, often including human settlements.

Four fully developed detached leaves of each plant species were placed separately in a Petri dish each on moist filter paper. Pieces of PDA (4 x 4 mm in size) with mycelium of tested isolate of *P. pseudocryptogea* on it developed for 7 days were placed with mycelium surface on the upper sides of the leaf lamina. The Petri dishes were covered and maintained at room temperature. In additional detached leaves assay five raspberry cultivars were tested for their reaction in inoculation with mycelia mats of *Phytophthora pseudocryptogea*. The assay was maintained in the same way as it was done with detached leaves of different plant species described above. Five leaves of each raspberry cultivar were used in the test. The diameter of developed spots on the leaves was measured 7 days after inoculation.

RESULTS AND DISCUSSIONS

Determination of *P. pseudocryptogea* specie

The obtained *Phytophthora* isolate was identified based on the sequence homology of the ITS region and determined as *Phytophthora pseudocryptogea* (100% of homology).

Morphology of colonies and asexual structures

The obtained isolate of *Phytophthora pseudocryptogea* formed stellate colonies with abundant aerial mycelium on V8 juice agar medium (Figure 1, a). The species is with heterothallic mating system and did not produced sexual structures in single culture. In non-sterile pond water *Phytophthora pseudocryptogea* formed small non-papillate predominantly ovoid (Figure 1 b, c) and more rarely obpyriform sporangia (Figure 1, d, e). Their dimensions were as follows: average length of 36.61±0.62 μm, average width of 26.91±0.40 μm, and length to width ratio 1.36±0.01.

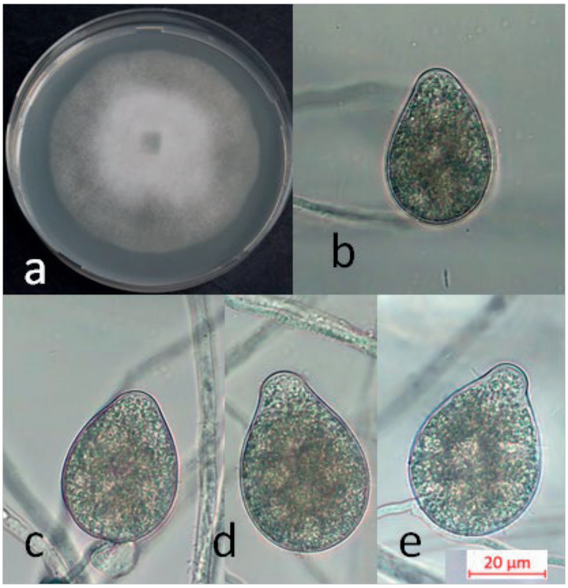

Figure 1. Morphology of the colonies and asexual structures of *P. pseudocryptogea*: a) 6 days culture of *P. pseudocryptogea* on V8 agar media; b)-e) sporangia formed in non sterile pond water after 36–48 h

Pathogenicity of *P. pseudocryptogea*

The leaves of the tested woody and shrubby plants show different responses on the inoculation with mycelium of *Phytophthora pseudocryptogea*.

The leaves of blackberry, linden and Japanese knotweed were not infected and there were no leaf spots observed (Figure 2).

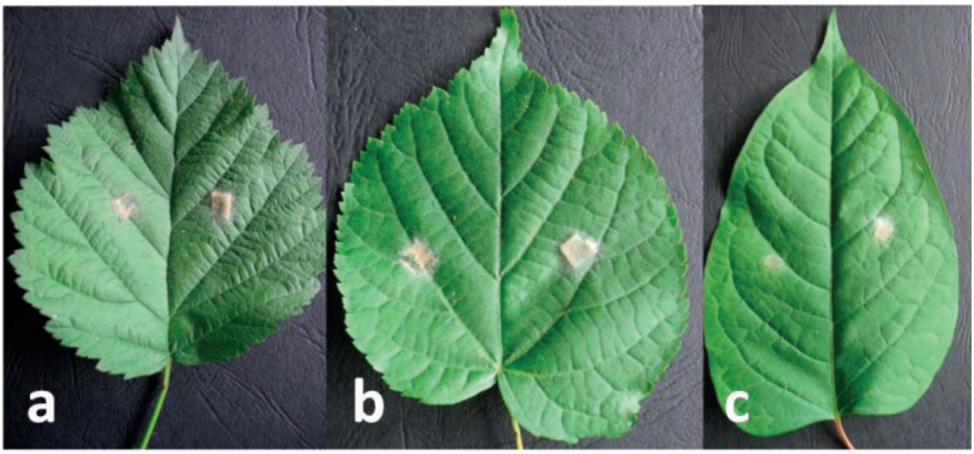

Figure 2. Inoculated detached leaves of blackberry (a), linden (b) and Japanese knotweed (c), inoculated with mycelium of *P. pseudocryptogea* 10 days after inoculation. No leaf spots developed

On the contrary very well formed large necrotic lesions developed on the inoculation points on the leaves of Turkey oak, sweet cherry and cherry laurel (Figure 3).

The successful inoculation of the leaves of the three plant species supposed that these plant species are to great extent potential hosts of the pathogen. The fact that the plants are broadly spread in ature and in the living areas amplified the risk of the distribution of *P. pseudocryptogea* and increased damages and losses in city green areas, nurseries and forestry.

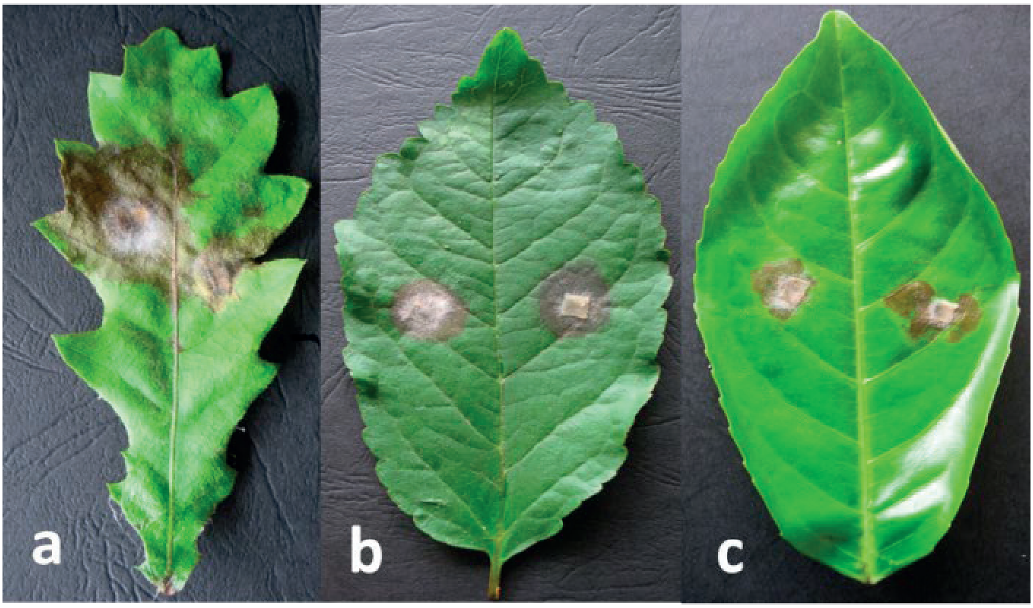

Figure 3. Inoculated detached leaves of Turkey oak (a), sweet cherry (b) and cherry laurel (c), inoculated with mycelium of *P. pseudocryptogea* 10 days after inoculation. Necrotic lesions appeared on the points of inoculation

The host range of *P. pseudocryptogea* is quite extensive. Among the plant species that are proven as hosts of *P. pseudocryptogea* are the following woody and annual plants: Pacific silver fir (*Abies amabilis*), kiwi fruit (*Actinidia chinensis*), strawberry tree (*Arbutus marina*) African daisy (*Arctotis acaulis*) thistle dyandra (*Banksia cirsioides*), sugar beet (*Beta vulgaris*), marri tree (*Corymbia calophylla*), cucumber (*Cucumis sativus*), squash (*Curcurbita pepo*), carrot (*Daucus* sp.), *Isopogon buxifolius*, walnut (*Juglans regia*), tomato (*Lycopersicon esculentum*), apple (*Malus pumila*), purple African nightshade (*Solanum marginatum*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*), Western Australian grass tree (*Xanthorrhoea preissii*) (Khaliq et al., 2019; Dalshed et al., 2020; Farr & Rossman, 2020; California Pest Rating Proposal for *Phytophthora pseudocryptogea*, 2020). The above mentioned list includes trees, annual plants, wild and endemic plants, and a large number of cultivated crops. *P. pseudocryptogea*, together with *P. cinnamomi*, *P. cryptogea*, *P. erythroseptica*, and *P.* sp. *kelmania* are reported to cause disease in pomegranate orchards of Iran. *P. pseudocryptogea* and *P.* sp. *kelmania* were isolated from root crowns of infected trees (Ghaderi & Habibi, 2021). *P. pseudocryptogea, P. acerina*, and *P. plurivora* are reported as associated with declining of common alder trees (*A. glutinosa*) in Italy. All three *Phytophthora* species are assumed as a serious threat to riparian alder ecosystems in Sardinia, based on their widespread occurrence and virulence (Seddaiu & Linaldeddu, 2020).

P. plurivora and *P. pseudocryptogea* were obtained using baiting and selective media from soil samples taken around symptomatic oak trees (*Quercus robur*) in Emirgan Grove, İstanbul. Both *Phytophthora* species were proven as pathogenic in inoculation tests on 2- to 3-year-old *Q. robur* and *Q. suber* seedlings. Thus it is accepted that *P. plurivora* and *P. pseudocryptogea* may play roles in the dieback of the oaks, including *Q. robur* and *Q. suber* (Kurbetli et al., 2022). *P. pseudocryptogea* is also supposed to play a role in decline of Holm oak (*Quercus ilex*) in Europe (Mora-Sala et al., 2018). According to us, the Turkey oak (*Quercus cerris*), sweet cherry (*Prunus avium*) and cherry laurel (*Prunus laurocerasus*) are reported as potential hosts of this pathogen for the first time.

Detached leaf bioassays of raspberry cultivars

Five raspberry cultivars were tested for their response to the inoculation of *P. pseudocryptogea* in detached leaf assays. Three of them are Bulgarian raspberry cultivars - Ljulin, Shopska alena and Balgarski rubin, and two of them are introduced - Meeker and Heritage. All five raspberry cultivars appeared to be infected by the pathogen. However, there was a slight difference in the response of the cultivars (Figure 4).

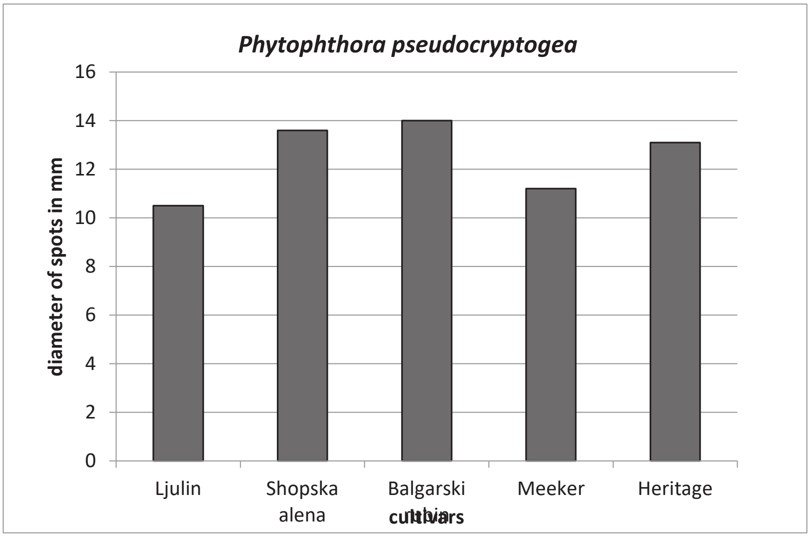

Figure 4. Response of raspberry cultivars to inoculation with *P. pseudocryptogea* in detached leaf assay

CONCLUSIONS

Phytophthora pseudocryptogea was isolated from a soil sample and was associated with the collar and root rot of raspberry in Bulgaria. It was shown to be pathogenic to Turkey oak, sweet cherry, and cherry laurel plants. Among the tested raspberry cultivars there were not found resistant ones, although a difference in the response to the infection was noticed among the cultivars.

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CROWN SHAPING AND PRUNING OF SWEET CHERRY TREES WHICH OPTIMIZE THE RATIO BETWEEN GROWTH AND FRUCTIFICATION

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Abstract

The vigour of the variety-rootstock association, the crown shape, the planting distance, the age of the trees, and the tree pruning pattern have been examined. The studies regarding the establishment of sweet cherry orchards, as well as the growth and fruiting of the 'Valerii Cikalov', 'Record', 'Ferrovia', 'Kordia', 'Regina', 'Stella', 'Skeena', 'Bigarreau Burlat', 'Lapins', 'Early Star', 'Samba', 'Black Star' sweet cherry trees grafted on the Prunus mahaleb L., 'MaxMa 14' and 'Gisela 6' rootstocks, in different combinations and planting distances, have been carried out in the Southern and Central fruit-growing area of the Republic of Moldova. High vigorous trees are used on non-irrigated soils in association with self-fertile varieties at optimal planting distances that ensure high yields. The sweet cherry trees, grafted on the semi vigorous rootstocks ('Gisela 6') and the moderate vigour rootstocks ('MaxMa 14') are used on fertile soils and irrigated at high densities. They can provide medium vigour of trees, which can be handled completely from the ground level, produce early average yields, with low pruning and fruit harvesting costs due to increased labour productivity. The pruning done during the growing period resulted in the vegetative growth of the trees and the formation of fruiting branches that produced better flower buds compared to the pruning done during the dormant period. The rootstock-variety association should be chosen in accordance with the direct task, the growing system and the applied technology.

Key words: *Cerasus avium, variety, rootstock, crown shape, density, cultivation system.*

INTRODUCTION

Sweet cherry orchards occupy more than 440 thousand hectares on the globe, and the world fruit production is about 2,3 million tons per year, of which 35% is in Europe. In the Republic of Moldova, the sweet cherry orchards occupy 4,100 ha, which yield over 10,000 tons per year. It is a rather modest yield in comparison with the average world yield (Cimpoies, 2018). During the last decades, the sweet cherry crop has worked up the market due to the rich assortment of high-quality self-fertile varieties, and the diversity of vegetative semi dwarfing and moderate vigor rootstocks. Currently, the sweet cherry growing implies mechanized high-density orchard systems with a high consumption of pesticides and fertilizers. Therefore, in response to these requirements, sustainable integrated culture systems have been successfully introduced in the Moldovan fruit growing sector (Babuc, 2012; Balan, 2019; Cimpoies, 2018).

The modernization of the sweet cherry crop is determined by the modern technological methods and technology used, the soil being the main production resource. The early maturation, the type of fruiting, the pruning methods and crown shaping, its diseases and pest's resistance, the planting density and the rootstock used are other factors that contribute to the realization of the biological production potential of this crop. The viable technologies used provide for increasing efficiency per surface unit by adopting modern cropping systems, which ensure quantitatively and qualitatively superior yields (Balan, 2015; Balan & Sarban, 2021). Trees of narrow fusiform crowns of 3.0-3.5 m in height, suitable for mechanized pruning both during the dormant period and the vegetation period at recommended for the orchards of tomorrow, in order to obtain high yields and fruits quality, as well as to reduces maintenance and harvesting manual labor costs (Bennewitz et al., 2010; Calabro et al., 2009; Long et al., 2014; Vercammen, 2002).

The creation of future systems in fruit growing is based on the results obtained as a result of theoretical and practical research carried out abroad and, in our country, namely:

Biological material. The various vigor of varieties and rootstocks allows good control of the tree growth. The large number of generative and vegetative rootstocks of different vigor offers the opportunity of cultivar-rootstock associations utilization in different cultivation systems and tree crown shaping, but also on less fertile soils (Gjamovski et al., 2016; Gyeviki et al., 2008; Long, 2003).

Currently, for the sweet cherry crop, it is recommended to use super-intensive orchards with trees grafted on semi dwarfing rootstocks (‘Gisela 5’), semi vigorous rootstocks (‘Gisela 6’, ‘P-HL-C’, ‘Krymsk 6’) and moderate vigor rootstocks (‘Krymsk 5’, ‘MaxMa 14’, ‘Piku 1’, ‘Piku 4’) planted at a distance of 3-4.5 x 0.5-2.5 m using trellises and wires, or individual poles installed in the year of the planting of the trees, or intensive orchards with trees grafted on the vegetative ‘Colt’, ‘CAB 6’ ‘Krymsk 5’, ‘MaxMa 14’, ‘Piku 1’ and ‘Piku 4’ rootstocks at a planting distance of 5-6 x 2.5-5 m, without any support for the fruit trees, and classic orchards with trees grafted on the vigorous rootstocks (Seedlings *Prunus avium*; ‘Colt’, ‘SL 64’) which are planted at a distance of 6-7 x 5-6 m, without any support for the fruit trees (Vercammen, 2002; Long, 2003; Aglar et al., 2016; Şarban & Balan, 2021; Usenik et al., 2010).

The tree crown shaping and pruning depends on the variety-rootstock association, climate (wind, temperature, relief, light, precipitation, etc.) and soil; but it certainly depends on tradition in order to maximize the biological potential of a sweet cherry orchard (Long et al., 2014; Ivanov & Balan, 2017; Balan et al., 2018). The high-volume globular crown shape, which is specific to extensive orchards, is progressively replaced by the fusiform crown shape. The tree crown shape must provide simplicity both in the process of crown shaping and, in the methods, and periods of branch pruning (Long et al., 2014).

Thanks to the large number of cultivar-rootstock associations and the planting distance, the sweet cherry tree can use all cultivation systems. As a result of the diversity of the existing biological material, numerous researches regarding the crown shape have been required. The following crown shaping systems can be mentioned: the crown shape for the extensive system - Rained Tiered Pyramid, Thinned Tiered Pyramid with semi-open center, Non-tiered Pyramid, Mixed Pyramid, Kym Green Bush - KGB etc.); for the intensive system – naturally improved low volume crown, improved slender spindle crown, Oblique-armed palmetto, Horizontal-armed palmetto, Loosely flattened palmetto, Vertical cordon, Drapeau Marchand, Tall Spindle Ax (TSA), Spanish Bush (SB), etc.; for the super-intensive system - Upright Fruiting Offshoot (UFO), Super Slender Ax (SSA) etc. (Musacchi et al., 2015; Sumedrea et al., 2014).

Currently, in the Republic of Moldova, all cultivation systems are used. That is why, the diversity of methods and technical opportunities, as well as the climatic conditions in the area, must be an object of study that will be the basis of the cultivation systems in the future. At the same time, it is considered appropriate to identify the theoretical elements that condition the effectiveness of the orchard (precocity, yields, fruit quality, etc.) and to study all the elements that define the cultivation systems and the relationships between them (Babuc, 2012; Aglar et al., 2019; Bujdosó & Hrotkó, 2012).

The aim of this research was to increase the productivity of a sweet cherry orchard (*Prunus avium* L.) by identifying highly productive cultivar-rootstock associations and establishing some strategies for tree crown pruning and shaping, and developing methods to maintain a balance between growth and fructification.

MATERIALS AND METHODS

The investigations regarding the development of systems of high-yielding sweet cherries cultivation were carried out on common semi deep, humus, clayey chernozem in the Southern, Central and Northern fruit-growing areas of the Republic of Moldova, caring out 6 stationary experiments. The orchard belonging to the "ProdCar" Ltd in the village of Negureni, the district of Telenesti, was planted in the spring of 2010 with sweet cherry trees of ‘Adriana’, ‘Ferrovia’ and ‘Skeena’ varieties grafted on ‘Gisela 6’ vegetative rootstock at a distance of 4 x 2 m. The trees had naturally improved low volume and improved slender spindle shaped crowns. The orchard belonging to the "Terra-Vitis" Ltd in the village of Burlacu, the district of Cahul, was established in the Southern fruit-growing area of the Republic of Moldova, in the spring of 2010, with sweet cherry trees of ‘Bigarreau Burlat’, ‘Ferrovia’, ‘Lapins’ varieties grafted on the vegetative ‘Gisela 6’ rootstocks, planted at a distance of 5 x 1.5 m, 5 x 2 m and 5 x 2.5 m. The trees had naturally improved low volume and improved slender spindle shaped crowns.

The researches at the "Vindex-Agro" Ltd in the village of Malaiesti, the district of Orhei, were conducted in the orchard established in the fall of 2003 which was planted with trees of the ‘Valerii Cikalov’ and ‘Record’ varieties grafted on *Prunus mahaleb* L. saplings; the planting distance was of 6x5m. The trees had naturally improved high volume shaped crowns. To achieve the intended purpose, the following pruning systems were studied: V1 - the maintenance and fruiting pruning during the dormant period (the control group); V2 - the maintenance and fruiting pruning during the vegetation period; V3 - the reduction cut done during the dormant period in 3- to 5-year-old branches; V4 - the reduction cut done during the vegetative period in 3- to 5-year-old branches. The same kind of works were done in the orchard established in 2011, with sweet cherry trees of the ‘Ferrovia’, ‘Kordia’, ‘Regina’ varieties grafted on the ‘Gisela 6’ rootstock, at a planting distance of 4x2.5m; the trees had naturally improved reduced volume and improved slender spindle shaped crowns.

Studies were carried out in the Central fruit-growing area of the Republic of Moldova, namely at the "StarAgro Group" Ltd in the village of Ustia, in the district of Criuleni, using the ‘Kordia’, ‘Regina’, ‘Stella’, ‘Ferrovia’ and ‘Skeena’ sweet cherry trees grafted on the ‘MaxMa 14’ rootstock. One of the orchards was planted in the fall of 2012, using trees of naturally improved reduced volume shaped crowns, planted at a distance of 5 x 3 m. Another orchard was established in the fall of 2015 with ‘Early Star’, ‘Samba’, ‘Black Star’ sweet cherry trees grafted on the ‘Gisela 6‘rootstock at a distance of 4 x 2 m.

Research methodology. The experiments were organized using four groups of eight representative trees each. The scheme was made according to the polyfactorial principle, with the placement of the groups through the randomized block system on 2 rows in the middle of the strip for each variety (Мойсейченко et al., 1994). The interdependence of the planting distance, crown shape, tree pruning system and time, as the basic factors that determine the tree fruiting, yield and fruits quality, was studied. Morphological, physiological and biochemical analyses, biometric measurements and the statistical processing of results were performed. The processed data are presented in average values over the 3-8 years of research. A 5% significance level test was used to compare the differences between the variants (Доспехов, 1985).

The orchard maintenance. The agrotechnical works in orchards were carried out in accordance with the regulations in force. In the orchards of the villages of Negureni, the district of Telenesti, the village of Burlacu, the district of Cahul and the village of Malaiesti, the district of Orhei, weather stations were installed to determine the state of the environment and the plants. The orchards of the "ProdCar" Ltd, the "Vindex-Agro" Ltd and the "StarAgro Group" Ltd were drip-irrigated, and Watermark transducers were installed at 20, 40 and 60 cm deep in each plot to monitor the soil moisture. Water was distributed by droppers fixed at a height of 40 cm from the ground in the direction of the row.

In the first two years after the planting of the trees, the soil was maintained as a worked field; during the following years, the soil between the tree rows was naturally or artificially grassed. The weeds on the 2-2.5 m wide strips of land between the rows were mowed when necessary and kept as mulches. The weeds were killed with herbicides applied along the rows, or mechanically weeded twice or three times using sensitive weeding equipment.

RESULTS AND DISCUSSIONS

The research conducted by the State Agricultural University of Moldova, on the increase of efficiency of sweet cherry orchards, by obtaining early bumper qualitative harvests, has led to the use of trees with preformed crowns thickened up to 90-100 cm which are planted more densely, to the development of methods to maintain a balance between growth and fruiting by minimizing pruning in the first years after planting, and to the utilization of inclined branches, irrigation and fertilization (Balan, 2015; Şarban & Balan 2021; Ivanov & Balan, 2017; Ivanov et al., 2019; Balan et al., 2022).
It is very important to know the natural potential of an ecosystem in order to implement a cultivation system which would lead to a larger and more qualitative crop, to lower maintenance costs and even to the improvement of the natural fertility of the ecosystem (Babuc, 2012; Balan et al., 2001; Sumedrea et al., 2014). Most soil types, with the exception of clay and anoxic soils, are suitable for growing sweet cherry trees, grafted on an appropriate rootstock (Cimpoieș, 2018). The traditional rootstocks such as Wild Cherry (*Prunus avium* L.), 'Mahaleb' (*Prunus mahaleb* L.), 'Sante Lucie' ('SL 64'), 'Mazzard' (*Prunus avium*) are still good for calcareous, rocky, dry or hilly soils. The vegetative 'Colt' (*P. avium* x *P. pseudocerasus*) rootstocks are used instead of the seedlings of Wild Cherry, 'Mahaleb' and 'Santé Lucie' on fertile and moist soils (Babuc, 2012).

The tree pruning works have developed considerably; researches have focused on the pruning during the vegetation period rather than on the pruning during the dormant period. The basic objective of pruning is to maintain the optimal parameters of the crown structure and the balance of growth and fruiting in order to control the development of a tree from the earliest stage to maturity.
Regarding the effect of the types of pruning on the average fruit production, according to the data presented in Table 1, it can be said that during the years 2012-2019, the largest crop was produced when the fructification pruning was done by reduction cuts in 3- to 5-year-old branches during the vegetative period (V4) with 46.8 kg/tree in the 'Valerii Cikalov' variety and 48.4 kg/tree in the 'Record' variety, followed by the fructification pruning done by reduction cuts in 3- to 5-year-old branches during the dormant period(V3) with 42.5 kg/tree in the 'Valerii Cikalov' variety and 45.4 kg/tree in the 'Record' variety. The fruiting and maintenance pruning during the dormant period (V1) and during the vegetation period (V2) led to a smaller crop compared to the fructification pruning done by reduction cuts in 3- to 5-year-old branches during the dormant period (V3) and during the vegetation period (V4). The obtained results prove the fact that the pruning type significantly influenced the fruit harvest recorded in the 'Valerii Cikalov' and 'Record' sweet cherries trees grafted on the 'Mahaleb' rootstock during the fruiting period.

Table 1. The yield of cherry trees depending on the variety and pruning type, kg/tree
('Mahaleb' rootstock, planting distance - 6 x 5m, the age of the trees - 10-17, *Vindex Agro* Ltd)

Pruning type	Years						Average yield (2012-2019)
	2012	2013	2014	2015	2016	2019	
'Valerii Cikalov' variety							
V1 (control)	20.1	25.3	28.5	38.9	57.6	68.5	39.8
V2	22.7*	27.9	32.9	41.7	58.2	65.6	41.5
V3	21.8	30.2*	31.3	42.5	58.0	71.3	42.5
V4	23.4*	33.4*	39.4*	49.7*	62.9*	71.7	46.8
'Record' variety							
V1 (control)	20.1	21.8	34.4	45.2	49.5	87.8	43.1
V2	22.3*	23.7	35.9	47.3	49.9	88.7	44.6
V3	19.2	25.7*	37.0	49.1	55.4*	85.9	45.4
V4	18.9	26.4*	41.4*	52.3*	57.1*	4.2*	48.4
LSD 5%	2.14	3.12	4.16	4.74	4.51	5.04	-

The 'Ferrovia', 'Kordia' and 'Regina' sweet cherry trees, grafted on the 'Gisela 6' rootstock and planted at a distance of 4 x 2.5 min the orchard with 0.4-0.5 t/ha, began to bear fruit in the 4th vegetation year, and in the 5th they yielded 4.6-5 t/ha, but the values are not statistically proved. Starting with the 3rd fruiting year, the harvest tripled, being statistically assured, amounting to 12.31-13.29 t/ha in the 'Ferrovia' variety and to 11.27-12.83 t/ha in the 'Kordia' variety. In the years 2017 and 2020, the fog, rain and cold during the flowering period had a negative influence on the harvest. The average yield during the first 7 years of the tree fruiting was of 8.19-8.31 t/ha in the 'Ferrovia' variety, 7.65-8.31 t/ha in the 'Kordia' variety, and 7.21-7.88 t/ha in the 'Queen' variety (Table 2).

Table 2. The productivity of cherry trees depending on the variety and crown shape, t/ha.
('Gisela 6' rootstock, planting distance - 4 x 2.5 m, naturally improved low volume crown, the age of the trees - 4-10, *Vindex-Agro* Ltd)

Variety	Years							Average yield (2014-2020)
	2014	2015	2016	2017	2018	2019	2020	
Naturally improved low volume crown								
Ferrovia	0.50	5.00	2.31*	7.90	10.88	3.73*	7.03	8.19
Kordia	0.40	4.60	11.27	7.50	7.60	3.81*	8.37*	7.65
Regina	0.50	4.80	10.38	7.80	7.60	12.37	7.01	7.21
Improved slender spindle crown								
Ferrovia	0.50	4.90	13.29*	793	11.78	12.94	6.82	8.31
Kordia	0.40	4.70	12.83	8.88*	8.94	13.32*	9.12*	8.31
Regina	0.40	5.00	11.89	7.57	11.29*	11.99	6.99	7.88
LSD 5%	-	0.845	0.529	0.82	0.675	0.315	0.783	-

The shape of the crown did not significantly influence the yield in the studied varieties, because the small volume crown shape has a favorable impact on the growth of the sweet cherry trees grafted on the Gisela 6 moderate vigor rootstock in a high density system (Long, 2003; Usenik et al., 2010; Balan et al., 2018). At the „ProdCar" Ltd, the 'Adriana', 'Ferrovia' and 'Skeena' sweet cherry trees, grafted on the 'Gisela 6', also came into fruit in the 4th year after their planting yielding 0.66-1.56 t/ha; in the 5th year they yielded 4.25-5 t/ha (Table 3).

In 2015, as the trees got older, the fruit crop tripled and amounted to 11.87-13 t/ha in the Adriana variety, 13.25-14.12 t/ha in the 'Ferrovia' variety and 16 t/ha in the 'Skeena' variety. In 2016, the sweet cherry harvest doubled compared to the previous year and amounted from 21.87-22.50 t/ha in the 'Adriana' variety to 26.25-28 t/ha the 'Skeena' variety.

Table 3. The yield of the sweet cherry trees according to variety and crown shape, t/ha.
('Gisela 6' rootstock, planting distance - 4 x 2 m, the age of the trees - 4-11, *ProdCar* Ltd)

Variety	Years								Average yield (2013-2020)
	2013	2014	2015	2016	2017	2018	2019	2020	
Naturally improved low volume crown									
Adriana	0.62	4.37	11.87	21.88	10.87	12.96	13.38	9.32	10.66
Ferrovia	1,12	4.87	13.25	4.75*	5.75*	5.22*	10.79	4.28*	12.51
Skeena	0.63	4.25	6.00*	6.25*	6.88*	7.58*	7.04*	6.65*	14.41
Improved slender spindle crown									
Adriana	0.87	4.50	13.00	22.50	10.75	14.82	13.13	10.82	11.29
Ferrovia	1.56	5.00	14.13	4.50*	12.70	15.39	13.54	6.64*	12.93
Skeena	0.38	4.38	6.00*	8.00*	4.00*	7.50*	7.42*	8.99*	14.58
LSD 5%	-	0.435	0.971	1.315	1.429	1.423	2.305	1.314	-

In 2017, the crop decreased remarkably and represented only 10.75-10.88 t/ha in the Adriana variety, 12.70-15.75 t/ha in the 'Ferrovia' variety and 14-16.79 t/ha in the 'Skeena' variety. The average crop was larger when in the Skeena variety the crown of which are of improved slender spindle shape (14.58 t/ha) followed by the 'Ferrovia' variety (12.93 t/ha). The smallest crop was produced by the 'Adriana' variety (10.65 t/ha) in the group in which the trees had naturally improved low volume shaped crowns. It is a medium-sized yield for a sweet cherry orchard with trees grafted on ‘Gisela 6’ rootstock (Ivanov & Balan 2017).

The researchers conducted at the „Terra-Vitis” Ltd included the analyses of the structure of the sweet cherry orchard not only from the point of view of the growth vigor of the rootstock and variety, but also of the planting distance. The density of the trees, in the first years of vegetation, did not influence the length and width of the crown. It is a well-known fact that when the distance between the trees in a row, from 1.5 m to 2.5 m, increases, the time to fill the area reserved for the crowns increases too.

The crowns of the ‘Bigarreau Burlat’ and ‘Ferrovia’ varieties joined in the direction of the row in the 4th year, and the ‘Lapins’ variety - in the 5th year of their vegetation, and reached the necessary parameters to capture the maximum solar energy to produce large harvests of qualitative fruits.

The trees began to bear fruit in the 4th year of vegetation and yielded from 0.32-0.4 t/ha, when planted at the distance of 5 x 2.5 m, to 0.67-1.07 t/ha when planted at the distance of 5 x 1.5 m (Table 4). In the 2nd year of fruiting, the largest statistically proved crop (5.48-6.82 t/ha) was produced by the trees planted at the distance of 5x1.5 m; the smaller crop (3.72-4.2 t/ha ha) - by the trees planted at the distance of 5x2.5 m. In the 3rd year of fruiting, the trees yielded 8.0-12.86 t/ha; in the 4th year - 14.62-20.07 t/ha. During the period of tree growth and fruiting, the sweet cherry crop was higher and statistically proved when the trees were planted at a smaller distance from each other. The trees the crowns of which are of improved slender spindle shape turned out to be the most productive, as compared to the trees the crowns of which are of naturally improved low volume shape, but these values are not statistically proved. During the growth and fruiting period, the ‘Bigarreau Burlat’, ‘Ferrovia’ and ‘Lapins’ cherry trees, grafted on the ‘Gisela 6’, produced an average yield of 8-10 t/ha.

With regard to the variety productivity, it is worth mentioning that the highest crop was recorded in the ‘Ferrovia’ variety planted at the distance of 5x1.5 m (18.94-20.07 t/ha), and the lowest crop, with the trees planted at the distance of 5 x 2.5 m (15.63-16.90 t/tree)in the 7th year of vegetation. The phenomenon can be explained by the difference in the number of trees per hectare between the two planting schemes.

It is also worth mentioning that the cherry trees grafted on the ‘Gisela 6’ rootstock and planted at high density can have a medium-sized crown, stimulate the early fruiting, increase pruning productivity and make it possible to gather fruit from the ground (Cimpoies, 2018; Ivanov & Balan, 2017); planting density is limited by the economic law of diminishing marginal productivity (Sumedrea et al., 2014).

Table 4. The yield of sweet cherry trees depending on the variety, planting distance and crown shape, t/ha. (‘Gisela 6’ rootstock, the age of the trees - 7, *Terra-Vitis* Ltd)

Variety	Planting distance, m	Naturally improved low volume crown				Improved slender spindle crown			
		Year 2013	Year 2014	Year 2015	Year 2016	Year 2013	Year 2014	Year 2015	Year 2016
Bigarreau Burlat	5 x 1.5	0.79	5.96*	11.41*	17.98*	0.71*	6.34*	12.21*	18.94*
	5 x 2	0.40	4.89	9.15	15.16	0.50	5.12	11.82	16.16
	5 x 2.5	0.32	3.72	8.00	14.62	0.40	4.17	10.20	15.63
Ferrovia	5 x 1.5	0.93	6.39*	12.72*	18.16*	1.07*	6.82*	13.82*	20.07*
	5 x 2	0.70	5.26	12.35	15.92	0.80	5.58	13.13	17.81
	5 x 2.5	0.56	4.45	9.88	15.37	0.64	5.02	12.89	16.90
Lapins	5 x 1.5	0.66	5.48*	12.28*	18.55*	0.67*	5.79*	11.86*	19.38*
	5 x 2	0.50	4.580	9.400	17.240	0.500	5.010	9.900	18.210
	5 x 2.5	0.40	3.720	9.888	16.152	0.400	4.200	9.520	17.536
LSD 5%		0.275	0.647	1.375	1.284	0.275	0.647	1.375	1.284

During the fruiting period, the yield of cherry orchards depends on the variety and the climatic conditions (Figure 1). Thus, in 2018 the ‘Ferrovia’, ‘Kordia’, ‘Regina’, ‘Skeena’ and ‘Stella’ sweet cherry trees, grafted on the ‘MaxMa 14’ rootstock, yielded from 4.18 t/ha in the ‘Ferrovia’ variety to 15.70 t/ha in the ‘Skeena’ variety. A significantly higher crop was produced by the ‘Kordia’ variety (10.94 t/ha), the ‘Skeena’ variety (15.70 t/ha) and the ‘Stella’ variety (9.13

t/ha). In 2019, the record harvest of 19.22-19.31 t/ha was produced by the 'Kordia' and 'Regina' varieties, and the lowest harvest - by the 'Ferrovia' (4.11 t/ha) and the 'Stella' varieties (7, 99 t/ha). In the 10th year of vegetation, the 'Regina' variety produced the highest yield (12.05 t/ha) as compared to the 'Ferrovia' (2.73 t/ha) and the 'Kordia' varieties (2.91 t/ha). Over the years, the 'Kordia' (11.06 t/ha), 'Regina' (12.35 t/ha) and 'Skeena' (11.31 t/ha) varieties produced an identical crop, while the 'Ferrovia' (3 .68 t/ha) and 'Stella' (8.81 t/ha) varieties produced a smaller crop.

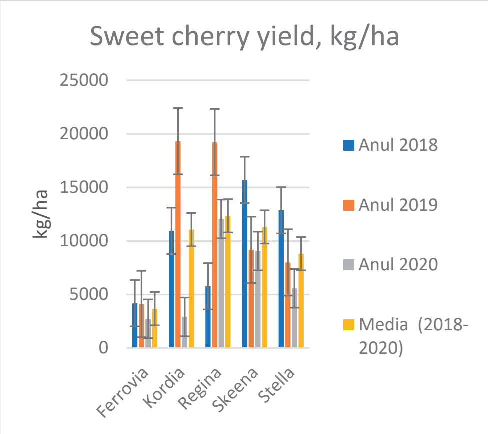

Fig. 1. The yield of sweet cherry trees, kg/ha. ('MaxMa 14' rootstock, planting distance - 5 x 3 m, naturally improved low volume crown, the age of the trees - 8-10, *StarAgroGroop* Ltd)

The 'Early Star' and 'Black Star' varieties, grafted on 'Gisela 6' rootstock, planted at a distance of 4x2 m started to bear fruit (2.9-3.7 t/ha) in the 4th year of vegetation, and the Samba variety - in the 5th year of vegetation (Table 5). In the 2nd year of fruiting, a higher crop was produced by the 'Samba' (16.82 t/ha) and 'Black Star' (10.75 t/ha) varieties; the lowest crop was produced by the 'Early Star' variety (7.01 t/ha). In the 4th year of fruiting, the 'Black Star' variety proved to be more resistant to late spring frosts, thus it yielded 9.87 t/ha. The 'Early Star' and 'Samba' varieties yielded only 3.62 -4.46 t/ha. In 2021, the crop of the 'Early Star' (9.45 t/ha) and 'Samba' (16.26 t/ha) varieties was significantly larger as compared to the 'Black Star' variety (5.50 t/ha). In the 5th year of fruiting, the crop amounted to 9.44-11.01 t/ha, being higher in the 'Early Star' variety. On average over 5 years, the most productive proved to be the 'Samba' variety which yielded 10.07 t/ha.

Table 5. The sweet cherry yield, t/ha.
('Gisela 6' rootstock, planting distance - 4 x 2 m, improved slender spindle crown, the age of the trees - 4-6, StarAgroGroop Ltd)

Variety	Years					Average yield
	2018	2019	2020	2021	2022	
Early Star	2.94	7.01	4.46*	9.45*	11.01	6.97
Samba	3.66	16.82*	3.62	16.26*	9.98	10.07
Black Star	0	10.75	9.87*	5.50	9.44	7.11
LSD 5%	0.992	0.874	1.013	2.372	1.284	-

Choosing the structure and the cultivation system of sweet cherry trees

The effective utilization of natural resources such as soils, light, slopes and land exposure cannot be done without establishing the optimal parameters of the geometric structure of an orchard, which determine the yield and quality of the fruit. In this context, it is appropriate to identify the biotic and abiotic factors that define the cultivation system, which corresponds to the biological production potential of the orchard and the economic interests. (Balan, 2009; Balan et al., 2008).

To establishment of an orchard should be based on the following principles: the geographical conditions and natural soil fertility, the relative vigor of the variety-rootstock association, the planting density, early large harvests, and simple crowns easily adaptable to partial mechanization. At the present, there are a lot of the sweet cherry varieties and rootstocks of different vigor. The crown of this crop can be easily and differently shaped, and it can also be grown on less fertile soils and sloping lands using all cultivation systems.

To describe the relationships between the parameters of the structure of the fruit plantation, the following formula was used

$$L = H \text{ tg } \varphi - H \text{tg } \alpha + B$$

The method provides for the determination of the distance between the rows of trees according to the height of the crown (H), the width of the bottom of the crown (B), the angle of inclination of the lateral surface of the crown to the geografical longitude (α) and latitude of the locality (φ). The method described by V. Balan, 1996 can be used to establish the geometric structure according to their variation in different geographical conditions.

When determining the optimal productive potential of the sweet cherry orchard, the data related to the 'Ferrovia' sweet cherry trees, grafted on 'Gisela 6' rootstock, and the 'Valerii Cikalov' sweet cherry trees, grafted on the 'Mahaleb', in different positioning combinations during the fruiting period were used (Table 6).

Table 6. The optimal productive potential of the cherry orchard depending on the crown structure (geographical latitude 47°, the angle of crown inclination 12°)

Distance between the rows, m	Width of the bottom of the crown, m	Hight of the crown, m	Level of soil covarage, %	Real volume of the crown, thousand m^3/ha	Crown surface, thousand m^2/ha	Production potential, %
'Ferrovia' variety, grafted on the 'Gisela 6' vegetative rootstock						
4	1.5	2.9	37.5	6.5	16.4	71.7
4	2.0	2.3	50.0	8.8	14.4	68.3
5	2.0	3.5	40.0	8.6	15.3	65.5
5	2.4	3.0	48.0	10.6	14.5	67.0
'Valerii Cikalov' variety, grafted on the 'Mahaleb' generative rootstock						
6	2.5	4	41.6	11.1	15.0	65.7
6	3.0	3.5	50.0	13.2	14.4	67.6

In order to determine the parameters of the orchard structure, the relationship between the height of the crown, the angle of inclination of the crown and the free area between the crowns in the neighboring rows were applied. In the 'Ferrovia' variety, in the tees 2.9 m in hight, the level of the soil coverage was 37.5%, and in the trees 2.3 m in hight, the level of soil coverage was 50%. At the distance of 5 m between the rows, the level of soil coverage was 40.0-48.0%. In the 'Valerii Cikalov' variety grafted on the 'Mahaleb' rootstock, the level of soil coverage was practically the same - 41.6-50.0%. The level of ground coverage increases with the decrease in the tree height.

In the 'Ferrovia' variety, the actual volume of the crown, at a distance of 4 m between the rows, was 6.5-8.8 thousand m^3/ha, and at a distance of 5 m between the rows, the volume was 8.6-10.6 thousand m^3/ha. In the 'Valerii Cikalov' variety grafted on the 'Mahaleb' rootstock, the volume increased significantly and amounted to 11.1-13.2 thousand m^3/ha. Thus, the crown volume gradually decreases with the increase in the density of the trees. The obtained data demonstrate that in high-density orchards, the actual crown volume reaches optimal values depending on the structure of the orchard during the first 3-4 years. The lateral surface of the crown was 14.4-16.4 thousand m^2/ha. The area of crowns in the orchard decreases with a decrease in the height of trees.

The value of the production potential of the orchard was calculated according to the efficiency coefficient of the orchard and the volumetric density coefficient of the lateral surface of the crown (Агафонов, 1983). The obtained calculations show that the value of the production potential of the orchard is higher in the orchards with a crown width of 1.5 m and constitutes 71.7%. The height of the trees is the basic element in the development of the cultivation system, because it modifies the technologies of crown pruning and shaping, determines the consumption of manual labor when pruning the trees and harvesting the fruit (Balan, 2009).

Comparing the data on the production potential of sweet cherry orchards with the data presented by other authors, it can be said that they are almost equal to those considered optimal, without changing the angle of inclination of the lateral surface of the crown to the vertical one. At the same time, when reducing of the height of the trees, a better lighting regime is created, which lead to the development of the fruit and the production of a large and qualitative harvests.

In conclusion, it has to be mentioned that the cultivation system for the sweet cherry trees is chosen depending on the variety-rootstock association, the ecological and technological conditions, which determine the productivity, the yield and the quality of the fruit.

It is also necessary to specify that crowns in a vertical plane up to 2.5-3 m high and 1.5-2 m wide make it possible to organize the orchard care, pruning and fruit picking at lower cost.

CONCLUSIONS

In order to achieve sustainable fruit production, it is necessary to identify the biotic and abiotic factors that define the orchard system of tomorrow. For the cultivation of sweet cherries, all sustainable integrated cultivation systems are successfully used, which are based on geographical conditions, the degree of natural soil fertility, the relative strength of the rootstock-varietal relationship, planting density, simple crowns, early and high yields in order to achieve high economic efficiency.

High vigor rootstock-varietal associations (Seedlings of *Prunus avium;* 'Colt', 'SL 64') are used on non-irrigated soils in association with self-fertile varieties at optimal planting distances that ensure high yields. Sweet cherry trees, grafted on the semi vigorous rootstocks ('Gisela 6', 'P-HL-C', 'Krymsk 6') and moderate vigor rootstocks ('Krymsk 5', 'MaxMa 14', 'Piku 1', 'Piku 4'), are used on fertile soils and irrigated at high densities, provides simple crown shapes, medium-sized trees, which can be managed from the ground level, early harvests, medium yield, reduced costs of tree pruning and fruit harvesting by increasing labor productivity.

The cultivation system for the sweet cherry crop is chosen according to the rootstock-varietal association, the ecological and technological conditions, which determine the productivity, the yield and the quality of the fruit.

The crowns in the vertical plane up to 2.5-3 m high and 1.5-2 m wide make it possible to organize the orchard care, pruning and fruit picking at lower cost.

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THE LIGHT REGIME AND THE STRUCTURE OF THE CROWN OF THE GOLDEN DELICIOUS REINDERS AND RED VELOX APPLE TREE VARIETIES

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Abstract

The given work relates to the study of the light regime and the structure of the vegetative composition in the Golden Delicious Reinders and Red Velox apple tree varieties. The solar regime in the crown of the apple trees of the studied varieties in the 4th year after their planting differs depending on the position of the sun in the sky, the distance from the ground and the length of the tree rows. The intensity of the solar radiation gradually increases from 9 o'clock till 13-15 o'clock, then decreases. The penetration of the solar energy into the crown increases from the base of the crown to the top of the tree. Based on the data obtained during the study, it can be asserted that the orchards in which trees have a height of 3.5-4.0 m and a crown width at the base of 1.0-1.2 m which decreases towards the top up to 0.8-1.0 m, form well-lit fruit-growing ecosystems, which receive in all areas of the vegetative ensemble more than 0.2 cal/cm^2 x min, i.e. as much as it is necessary for the photosynthesis process. The volume of the crown of the studied varieties depends on the vigour of the variety and the size of the trees. It provides light penetration to all elements of the crown, which allows.

Key words: *apple variety, light intensity, tree crown.*

INTRODUCTION

The apple tree, being a crop adapted to various ecological conditions, occupies over 60% of the total of fruit trees in the orchards of the Republic of Moldova, with a share of over 70% of fruit production; it is profitable and ensures high profits. One of the main functions of the orchard system is the conversion of solar energy into chemical energy bound in fruit. Light interception studies have shown that, in order to obtain high yields, the crown of trees must allow the interception of at least 70% of the available light radiation (Bîlici I., 2018). At the same time, the optimal level of soil coverage by the projection of the tree crown into full productivity turned out to be 65-70% of the nutritional surface. (Babuc V., 2000; Cimpoieș Gh. et al., 2001). However, this cannot be achieved in modern orchards. (Dadu C., 2000) the vegetative growth and the stem development is determined by biological (variety, rootstock, disease and pest resistance) and technological factors (the number of fruit, food and water supply), which condition the development of the physiological processes (Balan V. et al., 2018). The influence of soil factors such as soil structure and fertility, limiting soil factors, and climatic factors such as solar energy, temperature, its maximum and minimum values, but especially the values recorded during the growing season (fruiting phenophases), as well as the amount of precipitation and others should not be overlooked. (Ștefan N., 1993). From the genetic point of view, the variety is the basic factor in determining the crop system and technology.

MATERIALS AND METHODS

The research was carried out at the "Elit Fruct" Ltd in the village of Cosernita, the district of Criuleni, between the years 2015 and 2019. The orchard was founded in 2015. Planting material of 2-year-old trees of the "Certificate" category with a crown base consisting of well-developed branches evenly distributed along the axis was used. The Red Velox and Golden Delicious Reinders grafted on M9 rootstock were studied. The spacing between rows was 3.2 m and between trees – 0.8 m, which corresponded to

3900 trees/ha. The grafting site was placed at a level of 15–20 cm above the ground. Before the orchard was planted, a tree support system was installed – monoplane, simple, made of reinforced concrete poles, with a height of about 4.0 m above the ground, and a metal wire installed at a height of 50 cm above the ground level, which was also used as a support for the irrigation system. In the first year of vegetation, five more metal wires were added. The first two wires were fixed at 80 cm above the ground and at 80 cm between each other, the others - at 160 cm, 240 cm and 320 cm above the ground, respectively. Four groups of trees, 8 trees each, were used in the experiment. The research wa carried out according to the general methodology for conducting experiments with fruit trees.

The distribution of the solar energy in the crown of the Red Velox and Golden Delicious Reinders apple trees was determined by the height of the sun relative to the horizon, the height of the trees, and the density of the crown at the base in the direction between the rows. The rows of trees were in a north-south direction.

RESULTS AND DISCUSSIONS

The light regime in the crown of the Golden Delicious Reinders apple trees, which have an improved spindle-shaped crown type, varies depending on the position of the parts of the crown in relation to the sun and the distance from the ground. Since the crowns of the trees closed along the row, daylight illumination was studied not only in the centre of the crown along the axis, but also in the area of crown closure (Table 1, Figure 1a).

At 9 o'clock, the lowest amount of solar radiation was recorded at the height of 0.5-1.5 m above the ground, in the western part of the crown (0.21- 0.26 cal/cm^2*min) and in the central part (0.24-0.29 cal/cm^2*min) of the tree rows.

The lowest amount of solar radiation was recorded at the height of 0.5-1.5 m above the ground, in the western part of the crown (0.21-0.26 cal/cm^2*min) and in the central part (0.24-0.29 cal/cm^2*min) along the rows of the trees. At the top of the crown, all parts were well lit (0.32-0.36 cal/cm^2*min).

Table 1. The light regime in the crown of the Golden Delicious Reinders variety trees, cal/cm^2*min (the planting year - 2015, "Elit Fruct" Ltd, July 2018)

Time of determination, hour	Solar radiation in open field	Axis side		
		the east side	the centre of the crown	the west side
9^{00}	0.43	0.31	0.28	0.25
11^{00}	0.82	0.72	0.51	0.50
13^{00}	1.07	0.89	0.57	0.72
15^{00}	1.12	0.69	0.69	0.92
17^{00}	0.89	0.58	0.68	0.70
Average	0.87	0.64	0.55	0.62

At 11 o'clock, the solar radiation level in the open field was twice as high (0.82 cal/cm^2*min) compared to the level recorded at 9 o'clock (0.43 cal/cm^2*min). At this time, the eastern part of the crown benefited from more solar energy (0.65-0.82 cal/cm^2*min) compared to the central part of the crown (0.31-0.62 cal/cm^2*min) and its western part (0.38-0.67 cal/cm^2*min).

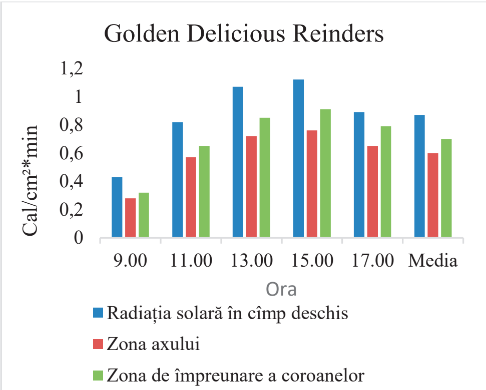

Figure 1a. The light regime in the crown of the Golden Delicious Reinders apple trees, cal/cm^2*min

At 13 o'clock, the sides of the crown were illuminated identically and received a large amount of solar energy (0.78-1.17 cal/cm^2*min) relative to the centre of the crown (0.42-1.13 cal/cm^2*minutes).

While the upper part of the crown received a high amount of direct solar radiation (0.88-0.96 cal/cm^2*min), at 0.5 m above the ground the central part of the crown received only 0.42-.53 cal/cm^2*min, and the western part - 0.62-0.79 cal/cm^2*min.

Thus, at noon, the crown of the Golden Delicious Reinders apple trees was well lit and the light intensity differed slightly in the eastern part compared to the western part, but the inner areas adjacent to the axis and the areas of crown interpenetration were less illuminated (Figure 1b).

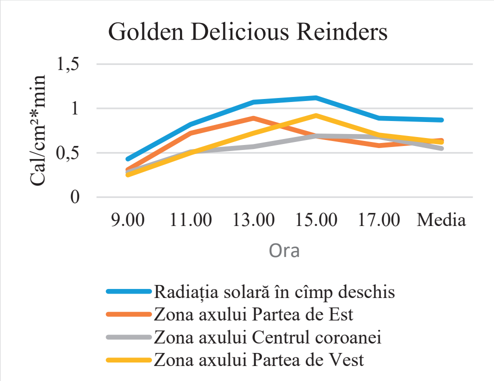

Figure 1b. The light regime in the area of the axis of the Golden Delicious Reinders apple trees, cal/cm^2*min.

At 15 o'clock, the level of the solar radiation in the open field was the highest (1.12 cal/cm^2*min), and the distribution of light in the orchard depended on the area of the crown.
Thus, the highest intensity of the solar energy was recorded in the area of the axis, in the western part of the crown (0.84-0.99 cal/cm^2*min), followed by the central area (0.63-0.93 cal/ cm^2*min) and the eastern part of the crown (0.45-0.95 cal/cm^2*min). In the area of the crown closure, the same pattern of crown illumination was recorded, in the sense that the western part was better illuminated than the central part and, to a lesser extent, the eastern part of the crown.
At 17 o'clock, in the region of the central axis, the western part of the trees in the orchard received the greatest amount of sunlight (0.69-0.79 cal/cm^2*min). The central and eastern parts of the tree rows were less illuminated - 0.62-0.75 cal/cm^2*min and 0.47-0.73 cal/cm^2*min, respectively.
The light regime in the crown of the Red Velox (Table 2.). apple trees proved that the variety had a decisive influence on the illumination of the crown.
The obtained experimental data show that a smaller amount of light penetrates into the lower part of the crown along the row of trees during the day compared to other parts of the crown. The illumination in the tree crown increases during the day as the height of the crown above the ground increases. At the same time, the illumination increases from morning to 13:00, when the eastern part of the crown is better lit, and the western and central parts of the crown are less lit. The intensity of the light the trees receive is slightly different at 13:00, In the afternoon, the western part of the crown receives more solar energy, and the central and eastern parts of the crown receives less energy.
The Red Velox variety forms much smaller leaves compared to the Golden Delicious Reinders variety which forms much larger leaves. Therefore, the light penetrates more easily into the crowns of the Red Velox apple trees, compared to the Golden Delicious Reinders variety, ensuring a rational lighting of the tree rows (Figure 2).
The light regime in the crowns of trees of the Red Velox variety indicates that the variety has a decisive influence on the illumination of the crown. Thus, the penetration of solar energy into the eastern and western parts of the crown in this variety is relatively identical to the Golden Delicious Reinders variety, but the centre of the crown is much better lit.

Table 2. The light regime in the crown of the Red Velox apple trees, cal/cm^2*min (the planting year 2015, "Elit Fruct" Ltd, July 2018)

Time of determination, hour	Solar radiation in the open field	Crown joining area		
		the east side	the centre of the crown	the west side
9^{00}	0.40	0.34	0.32	0.28
11^{00}	0.81	0.67	0.67	0.61
13^{00}	1.05	0.89	0.81	0.85
15^{00}	1.10	0.82	1.00	1.00
17^{00}	0.87	0.69	0.84	0.84
Average	0.85	0.68	0.73	0.72

At 9 o'clock, the Red Velox variety, both in the area of the central axis and in the crown closure, received 0.32-0.38 cal/cm^2*min or 80-95% of radiation, and at 11 o'clock - 0.60-0.67 cal/cm^2*min or 74-83%. At 13 o'clock, when the sun was at its zenith, the central part of the crown received 0.76-0.81 cal/cm^2*min or 72-77% of the total radiation. In the afternoon, the light intensity was quite high (0.87-1.10

cal/cm^2*min) and the central part of the crown was lit very well. Thus, at 15 o'clock, the illumination in the central part of the crown was 0.71-1.0 cal/cm^2*min or 65-91%, and at 17 o'clock, the centrl part of the crown received 0.68-0.84 cal/cm^2*min or 78-96% of the total radiation.
Therefore, the light penetrated more easily into the crowns of the Red Velox apple trees, compared to the Golden Delicious Reinders variety, and ensured a rational lighting of the tree row (Figure 2).

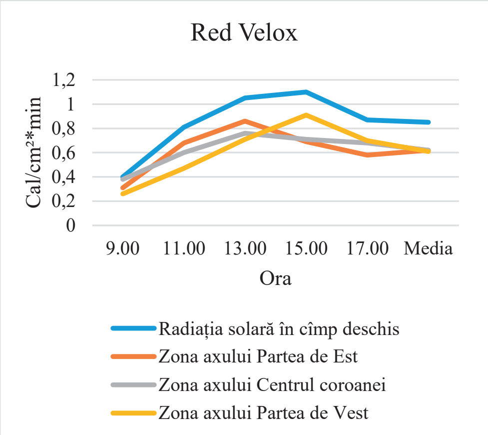

Figure 2. The light regime in the axis area of the Red Velox apple tree, cal/cm^2*min

Analysing the lighting regime in the apple tree orchards of the Golden Delicious Reinders and Red Velox varieties, it can be argued that in continuous rows oriented from north to south, the crown height of which at the base does not exceed 120 cm, the problem of self-shading does not persist, even if the height of the trees reaches 3.5-4.0 m. The intensity of the solar radiation received by the crown of the apple trees in the 4th year after their planting changes during the day depending on the height of the crown from the ground, as well as the solar radiation on open ground. The conducted studies aimed at establishing the level of illumination in the new varieties introduced into intensive horticulture in the Republic of Moldova will become the basis for establishing solar energy reserves to increase the photosynthesis yield in modern gardens.

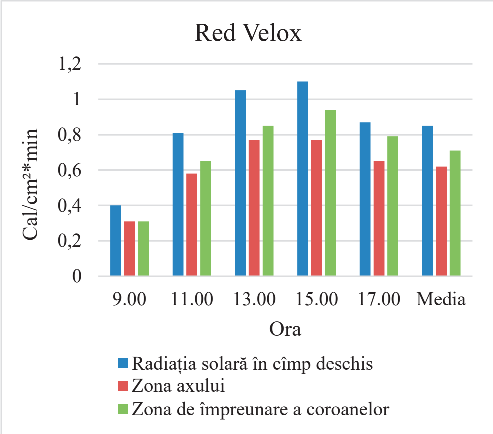

Figure 3. The light regime in the crown of the Red Velox apple trees, cal/cm^2*min

The vegetative growth of the studied varieties is expressed quantitatively in the amount of accumulated annual vegetative growth, crown height and width, crown surface and volume, as well as the degree of soil coverage by the shadow of the tree crowns (Figure 3.). The height of the crown of the studied varieties was 256-265 cm in the 3rd year after their planting. The dwarf variety Red Velox recorded the lowest value of the crown height, namely 256 cm.
The width of the crown at the base recorded maximum values (105-125 cm) admissible in relation to the planting distance of the trees in a row (80 cm). The width of the crown at the top depends on the growth vigour of the variety and varied between 25 cm in the Red Velox variety, and 50 cm in the high-vigour Golden Delicious Reinders variety.
In conclusion it must be said that the volume of the crown in the studied varieties depends on the vigour of the variety and the size of the trees (Table 3.). It provides light penetration to all elements of the crown, thereby creating the necessary conditions for the growth of fruit branches in order to obtain the highest possible yield.

Table 3. The structure of the crown of apple trees according to age and the biological characteristics of the variety (the planting year – 2015, "Elit Fruct" Ltd, 2017–2019)

Variety	Crown hight, cm	Crown width, cm		Degree of soil coverage by the shadow of the tree crowns, %	The lateral surface of the crown, thousand m^2/ha	Crown volume, m^3	
		at the basis	at the top			tree	ha
the year 2017, 3-year-old trees							
Red Velox	256	105	25	32.8	20975	1.6	6249
Golden Delicious Reinders	265	115	50	35.9	22654	2.2	8593
the year 2019, 5-year-old trees							
Red Velox	285	130	45	40.6	24021	2.5	9765
Golden Delicious Reinders	333	130	70	40.6	28748	3.3	12889

The degree of soil coverage by the shadow of the tree crowns which received solar energy reached the values of 32.8-35.9%. The difference in the use of the nutrition surface of the trees was insignificant, since the distance between the rows and within the row were optimal for the apple varieties grafted on the M9 rootstock in high density orchards.

Using the data on the tree vigour, the crown volume was calculated and related to the surface unit. The lateral crown surface of the 3-year-old apple trees was 20975-22654 m^2/ha, and the crown volume was 1.6-2.2 m^3/tree and 6249-8593 m^3/ha, respectively.

Along with the growth and development of the trees, the parameters of the crown also increased. In 5-year-old trees, the height of the crown was 285-333 cm, depending on the variety, and the width of 130 cm stayed constant in both varieties studied (the distance of 3.2 m between rows does not allow wider crowns). The width of the crown at the top varied from 45 cm in the trees of the Red Velox variety, to 70 cm in the trees of the Golden Delicious Reinders variety. The degree of soil coverage by the shadow of the tree crowns reached the optimum level possible for a row spacing of 3.2 m and was 40.6% for both varieties.

In 2019. the lateral surface of the crown (24021-28748 m^2/ha) increased significantly compared to 2017 achieving optimal values for such orchards. The crown volume, which depends on the crown area, also increased significantly, reaching 2.5-3.3 m^3/tree and 9765-12889 m^3/ha, respectively. In 2019, the crown area and volume were greater in the Golden Delicious Reinders variety compared to the Red Velox variety. Thus, in the 5th year of vegetation, the trees of the studied varieties reached the optimal surface and crown volume, which characterizes the productive potential of the orchard.

CONCLUSIONS

The intensity of the solar radiation gradually increased from 900 to 1300-1500 and then decreased; the penetration of the solar energy into the tree crown increased from the base of the crown to the top of the tree. In the first half of the day, the leaves in the eastern part of the continuous rows were better illuminated; in the second half of the day, the western part of the rows were better illuminated. The apple trees of the studied varieties grafted on the low vigour M9 rootstock, planted at a distance of 3.2 x 0.8 m and the crown of which are of improved slender-spindle shape, of a height of 3.5-4.0 m and a width of 1.0-1.2 m at the base and 0.8-1.0 m at the top, form well-lit orchards, which receive in all areas of the crown more than 0.2 cal/cm^2*min, i.e. the necessary amount for the photosynthesis process. The light intensity in the crown of the Red Velox variety is higher because these trees form much smaller leaves compared to the other varieties.

Along with the growth and development of the trees, the parameters of the crown also increase. In 5-year-old trees, the height of the crown is

285-333 cm, depending on the variety, and its width of 130 cm remains constant in both varieties which have been studied.

The width of the crown at the base of the tree recorded maximum values admissible (105-125 cm) in relation to the planting distance of the trees in a row (80 cm). The width of the crown at the top depends on the growth vigour of the variety and varied between 25 cm in the Red Velox variety, and 50 cm in the high-vigour Golden Delicious Reinders variety.

It has to be mentioned that the volume of the crown in the studied varieties depends on the vigour of the variety and the size of the trees. It provides light penetration to all elements of the crown, thereby creating the necessary conditions for the growth of fruit branches in order to obtain the highest possible yield.

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PRODUCTIVITY OF THE IDARED APPLE VARIETY DEPENDING ON THE CROP LOAD AT THE FRUIT THINNING

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Abstract

The experimental plot is placed in the orchard "Codru-ST" Ltd. founded in 2006. The study subject of the experience was Idared apple variety grafted on M 9 rootstock. The plant spacing is 3.5 x 1.2 m. The research was conducted during the period of 2014-2017 years. The active substances applied were NAD (Geramid-New), NAA (Dirager) and BA (Gerba 4 LG), using different doses and different thinning periods. During the research, such indicators as the number and average weight of the fruit, the yield per tree and per unit area and the average fruit diameter were studied. It was established that, the spray with Geramid-New in dose 1.2 l/ha when 80% of the petals have fallen + 2-3 days, Dirager in dose 0.2 l/ha when the king fruit diameter was 8-9 mm, and Gerba 4 LG in dose 2.0 l/ha when the king fruit diameter was 10-12 mm have a significant effect on the number of the fruits, average weight, yield and fruits diameter.

Key words: *Growth regulators, thinning, average weight, yield, fruits diameter.*

INTRODUCTION

The Idared variety is one of the most cultivated red apple varieties in the Republic of Moldova (Babuc et al., 2013; Balan & Vămășescu, 2011; Cimpoieș, 2012; Peșteanu, 2015). The Idared variety flowers abundantly and regularly on all types of fruiting branches forming high yields, but in some years of lower quality (Ambrozic Turk & Stopar, 2010; Peșteanu, 2015). In order to exclude this negative phenomenon, it is recommended to carry out the reduction of the fruit load by different thinning methods, (manual, chemical) with different growth regulators and different treatment doses (Greene, 2002; Ilie et al., 2016; Peșteanu, 2013; Peșteanu, 2015; Peșteanu & Calestru, 2015; Peșteanu & Calestru, 2020)

Therefore, at the initial stage, in apple orchards the fruit load of the trees is optimized by chemical method and then, if necessary, the number of fruits is corrected by manual method depending on the degree of development and physiological condition of the plants (Balan & Vămășescu, 2013; Basak, 2004; Peșteanu, 2015).

The chemical reduction of the reproductive organs is also studied by many researchers for various apple varieties, but no such research has been carried out for Idared, as it is grown only in CIS countries. To solve this problem, it is necessary to study the use of different growth regulators whose active ingredient are NAD, ANA and BA based products, which allow to perform treatments from petal fall until the central fruit in the inflorescence has a diameter of 15 mm (Balan & Vămășescu, 2013; Basak, 2004; Ilie et al., 2016; Peșteanu, 2013; Peșteanu & Calestru, 2017).

MATERIALS AND METHODS

The experiments on which the PhD thesis is based were organized in the period 2014-2017, in the intensive apple orchard of the "Codru-ST" Ltd. company, planted near the village of Păulești, Călărași district. The plantation was founded in autumn 2006, with one year old trees.

The Idared variety grafted on rootstock M9 was taken as the object of study. The crown was trained according to the improved thin spindle system, planting distance 3.5 x 1.2 m.

To determine the fruit thinning efficiency of Idared trees, growth regulators based on NAD, ANA and BA, produced by 'L.Gobbi' SRL, Italy, were used and the following experiment was set up (Table 1).

The research was carried out according to the general methods of carrying out experiments with fruit species both in the field and the laboratory.

Table 1. Experimental scheme for thinning method and treatment dose of apple trees with growth regulators

Variants of the experiment	Activ ingredient	Treatment dose, l/ha	Aplication
Without thinning (control)	-	-	-
Hand thinning	-	-	Manual thinning after physiological fall, when the central fruit reaches 15-20 mm in diameter.
Geramid New	NAD (44.8 g/l)	1.2	By spraying, at 80% petal fall +2-3 days, when the central fruit reaches 4-7 mm in diameter.
		1.5	
		2.0	
Dirager	ANA (37g/l)	0.2	By spraying, when the central fruit reaches 8-9 mm in diameter
		0.3	
		0.4	
Gerba 4 LG	6BA (41g/l)	2.0	By spraying, when the central fruit reaches 10-15 mm in diameter.

Trees were treated with portable sprayers during windless hours, in the morning, above +18°C. The amount of solution per tree was 0.4-0.5 liters, based on the number of trees per unit area and the recommended water quantity of 1000 l/ha.

Records for determining the number of fruits, average weight of a fruit, yield per tree and per unit area, and fruit diameter were established at harvest time. The results were reported to the control.

RESULTS AND DISCUSSIONS

The number of fruits on a tree results from the number of flowers and the setting coefficient depending on how the fruit load is managed and the dose applied on each individual growth regulator.

The data in Table 2 show that the number of fruits was directly correlated with the factors taken into account in the study. A higher number of fruits in Idared trees was recorded on the variants under study in 2016. In 2014-2015 the number of fruits was at the level of the multi-year average on the variants under study, except for the variant without thinning. In this variant, in 2014 a higher number of fruits was recorded (210 pcs/tree), and in 2015 there was a clear influence in the crown of the alternation of fruiting (87 pcs/tree), which finally influenced the multiannual average index (157 pcs/tree).

In 2017 the difference between years in terms of the number of fruits on a tree shows lower values of the index under study, based on the higher number of fruits in the previous year (2016).

Table 2. Influence of growth regulators and treatment dose on the number of fruit in the crown of Idared apple trees, pcs/tree

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without thinning (control)	-	210	87	258	73	157.0
Hand thinning	-	110	113	116	110	112.2
Geramid New	1.2	128	136	140	117	130.5
	1.5	110	117	113	89	107.2
	2.0	94	87	90	80	87.7
Dirager	0.2	116	113	124	101	113.5
	0.3	95	92	101	80	92.0
	0.4	74	77	85	76	78.0
Gerba 4 LG	2.0	96	100	104	89	97.2
	2.5	69	74	80	71	77.5
	3.0	54	66	70	51	60.2
Average	-	105.1	96.5	116.4	85.2	-

The number of fruits on a tree also varies significantly depending on how the fruit load is managed during the research. In the no thinning variant the number of fruits per tree after the multi-year average was higher (157 pcs/tree) compared to the manual thinning variant (112.2 pcs/tree). In the variants with chemical thinning, the index under study was correlated with the product used for thinning. Depending on the way of the fruit load management, during the research an variable number was scored in the control variant, without thinning, when partial alternation of fruiting is clearly observed, i.e. in one year (2014; 2016) a high number of fruits in the tree and in another very low. The number of fruits in the manual thinning variant was constant throughout the research and did not vary essentially from the multi-year average value, as the aim was to keep 110-115 pcs/tree in the crown.

The growth regulators used in chemical thinning of reproductive organs during the research had its contribution on the average number of fruits in trees. A higher number of fruits per tree was scored when treated with the growth regulator Geramid New (87.7-130.5 pcs/tree) compared to the product Dirager (113.5-78.0 pcs/tree). The lowest number of fruits per tree was obtained when using the growth regulator Gerba 4 LG (97.2-60.2 pcs/tree). The different number of fruits in Idared apple trees can be explained by the action of each active substance on the blocking of seed development in the seed chamber of the fruit and the climatic conditions that were recorded during the treatment period.

Since it is known that Idared belongs to the category of varieties in which the reproductive organs are easily chemically thined, different treatment doses from those recommended for apple cultivation in the conditions of the Republic of Moldova were also studied.

If, for example, the average number of fruits in the years 2014-2017 in the variant Geramid New 1.2 l/ha was 130.5 pcs/tree, then in the variant Geramid New 1.5 l/ha the value of this index was 107.2 pcs/tree, an approximate value with the manual thinning variant. Increasing the treatment dose of Geramid New to 2.0 l/ha essentially decreased the number of fruit in the trees, the index in the study being 87.7 pcs/tree.

The highest value in the variants where Dirager was applied was recorded in the variant treated with 0.2 l/ha. In the case of that variant, an average of 113.5 pcs/tree remained in the crown of the trees, and in the variants with increased treatment dose, the number of fruits per tree was decreasing. Thus, the Dirager 0.3 l/ha variant had 92.0 pieces of fruit per tree, while the Dirager 0.4 l/ha variant had 78.0 pieces or a decrease compared to the Dirager 0.2 l/ha variant by 19.5 and 31.3% respectively.

In the case of the variants treated with the growth regulator Gerba 4 LG there is a major decrease compared to the variants treated with Geramid New and Dirager.

The lowest number of fruits per tree when treated with Gerba 4 LG was obtained in the 3.0 l/ha (60.2 pcs/tree) dose variant, then higher values of the index in the study were recorded in the 2.5 l/ha (77.5 pcs/tree) dose variant. A higher number of fruits in the Gerba 4 LG treated variants was scored in the 2.0 l/ha dose (97.2 pcs/tree), but clearly lower in the Geramid New 1.2 l/ha, Geramid New 1.5 l/ha and Dirager 0.2 l/ha variants.

The average weight of a fruit as an indicator of quality (Table 3), varied greatly over the years studied in Idared trees. The highest average weight of a fruit was recorded in 2017 (201.3 g) and the lowest in 2016 (179.4 g). During 2014 and 2015 the average weight of a fruit recorded average values, constituting 185.1 and 191.4 g, respectively. This indicator largely correlates with the number of fruit obtained per tree and the weather conditions in the reference year.

Table 3. Influence of growth regulators and treatment dose on the average weight of a fruit in the crown of Idared apple trees, g

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without thinning (control)	-	103.9	189.7	95.5	220.1	152.3
Hand thinning	-	172.7	169.1	167.5	172.0	170.3
Geramid New	1.2	157.4	155.7	151.3	169.5	158.5
	1.5	174.3	169.5	186.0	192.1	180.5
	2.0	195.8	199.4	195.3	201.3	197.9
Dirager	0.2	168.7	170.9	158.3	191.7	172.4
	0.3	198.1	193.5	191.6	205.1	197.1
	0.4	217.3	215.1	199.7	216.3	212.1
Gerba 4 LG	2.0	194.4	192.3	194.1	195.7	194.1
	2.5	227.3	222.6	207.7	218.3	218.9
	3.0	230.4	228.0	226.8	232.1	229.3
Average	-	185.5	191.4	179.4	201.3	-

The average weight of a fruit also changes under the influence of the fruit load management method. Lower values of the given index were recorded in the variant without thinning (152.3 g) compared to the other variants (158.5-229.3 g). The essential discrepancy in the control variant on the studied index is explained by the different number of fruits per year of research. A lower fruit weight was obtained in 2015 and 2016 constituting 103.9 and 95.5 g, respectively, with very high fruit values recorded in 2015 (189.7 g) and 2017 (220.1 g). This phenomenon was recorded as a result of the alternation of fruiting, which in Idared is not as pronounced as in trees of other varieties and carries a partial effect. In the variant with manual thinning we recorded an average weight (170.3 g), which has balanced values throughout the research.

The products used in the chemical thinning of fruit also influenced the index in the study by recording different average weights of a fruit depending on the active substance used.
Comparing the average weight of a fruit according to the growth regulators used in chemical thinning, it was found that treating the trees with Geramid New 1.2 l/ha resulted in a value of 158.5 g, i.e. a decrease compared to the manual thinning variant by 6.9%. In the Geramid New 1.5 l/ha variant, the index studied increased to 180.5 g and in the 2.0 l/ha dose to 197.9 g. The increase in mean fruit weight correlated with the number of fruits obtained per tree on the variants studied.
When using Dirager and Gerba 4 LG growth regulators in chemical thinning, the average weight of a fruit at different treatment rates shows an higher increase compared to the Geramid New treated variants. Thus, if the average weight of a fruit in the last Dirager 0.2 l/ha variant was 172.4 g, then in trees treated with the 0.3 l/ha dose, the index in the study increased and amounted to 197.1 g. Higher values were obtained in the Dirager 0.4 l/ha variant (212.1 g).
The growth regulator Gerba 4 LG had a more visible action on the average weight of a fruit and recorded lower values in the variant with the 2.0 l/ha dose (194.1 g), then in ascending order the 2.5 l/ha dose (218.9 g) was placed and the fruits with higher mass were recorded in the variant with the 3.0 l/ha dose (229.3 g).
Fruit yield is the main index by which the effectiveness of the technological elements applied in apple cultivation can be assessed.
Fruit yield in apple trees is a complex characteristic that has a bearing on the method of management fruit load, how much product was applied at thinning and how these technological elements interacted with environmental factors.
The data in Table 3 show that the above-mentioned factors had an impact on fruit production in a tree.
The fruit production of a tree depends on the number of fruits in the crown and the average weight of a fruit. Higher fruit production in trees with different methods of fruit load management was obtained in 2016 compared to 2014 and 2015. In 2017, this index recorded lower values due to the above-mentioned factors.
The method of reducing of the fruit load during the research influenced the studied index, recording higher values in the Geramid New 1.2 l/ha variant with 20.58 kg/tree, then decreasing was the control variant, without thinning (19.75 kg/pom). If in the previous variant the gap during the research on fruit production was not big enough, then in the control variant we register lower values of the given indicator in 2015 and 2017 as in the case of the number of fruits.
An approximate value with the control variant, without thinning, was recorded for the Dirager variant 0.2 l/ha (19.6 kg/tree). The manual thinning variant recorded yields on a par with the Dirager 0.2 l/ha variant, constituting 19.11 kg/pom and 19.46 kg/tree respectively. The multi-year average yield per tree was recorded in the Geramid New 1.5 l/ha growth regulator treatment - 19.27 kg/tree and Gerba 4 LG 2.0 l/ha - 18.87 kg/tree.
Fruit yield per tree in the other variants was lower compared to the previous variants.
The fruit production in the crown of the trees was also influenced by the dose of product applied during the period of chemical thinning.
In Idared trees, higher fruit yields were obtained in the crowns of the variants treated with the growth regulator Geramid New at the doses of 1.2 l/ha (19.27 kg/tree) and 1.5 l/ha (20.58 kg/tree), which were higher or approximately the same as those obtained in the variant with manual thinning. Increasing the dose of product administered to 2.0 l/ha, decreased the fruit yield in the trees compared to the previous variants by 15.7 and 10.0%, respectively.
Within the variants treated with the Dirager growth regulator, fruit yield within a tree in the 0.2 l/ha treated variant where the index taken in the study recorded higher values compared to the Dirager 0.3 l/ha and 0.4 l/ha variants, by 7.3 and 15.2%, respectively.
Treatments with Gerba 4 LG regulator reduced fruit yield in trees more than Geramid New and Dirager. The yield of Idared trees ranged from 13.80 to 18.87 kg/tree. The value of this index for Gerba 4 LG 2.0 l/ha was higher than for Gerba 4 LG 2.5 l/ha and amounted to 18.87 compared to 16.06 kg/tree, i.e. an increase of

4.9%. In the case of Gerba 4 LG 3.0 l/ha the yield per tree was the lowest (13.80 kg), i.e. a 26.9% decrease in the index in the study.

Fruit yield per unit area did not differ much from that obtained from one tree because the number of trees in the variants studied was identical.

Averaged over the fruiting years, the highest fruit production was obtained in 2016 (45.2 t/ha), then decreasing in 2014 (43.23 t/ha), 2015 (42.92 t/ha) and lower values of this index were recorded in 2017 (40.09 t/ha).

The method of reducing of fruit load influenced differently on fruit production per unit area. Thus, if on average in the fruiting years (2014-2017) in trees of Idared variety in the variant Geramid New 1.2 l/ha fruit production amounted to 49.36 t/ha, in the control variant, without thinning, the value of the studied index was 47.03 t/ha. Theoretically, it would persist the hypothesis that the fruit yield would be higher in the variant without thinning, but based on the studies we record diametrically opposite results, which is explained by alternate fruiting of trees of the given variety in 2015-2017.

Table 4. Influence of growth regulators and treatment dose on fruit production in the crown of Idared apple trees, kg/tree

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without thinning (control)	-	21,82	16,50	24,64	16,06	19,75
Hand thinning	-	19.00	19.10	19.43	18.92	19.11
Geramid New	1.2	20.14	21.17	21.18	19.83	20.58
	1.5	19.17	19.83	21.00	17.09	19.27
	2.0	18.40	17.35	17.57	16.10	17.35
Dirager	0.2	19.56	19.31	19.63	19.36	19.46
	0.3	18.81	17.80	19.35	16.41	18.09
	0.4	16.08	16.56	16.97	16.44	16.51
Gerba 4 LG	2.0	18.66	19.23	20.18	17.41	18.87
	2.5	15.68	16.47	16.61	15.50	16.06
	3.0	12.44	15.05	15.87	11.84	13.80
Average	-	18.16	18.03	19.31	16.81	-

Identical values of the index taken in the study with the manual thinning variant (45.5 t/ha) were recorded in the chemical thinning variants treated with Geramid New at 1.5 l/ha (45.88 t/ha), Dirager 0.2 l/ha (46.50 t/ha) and Gerba 4 LG (44.92 t/ha). In the other variants this index was much lower compared to the manual thinning variant. The treatment dose for chemical thinning in the growth regulators taken in the study had a direct influence on the fruit yield calculated per unit area. So, for example, the fruit yield in case of treating trees with the growth regulator Geramid New 1.2 l/ha was 49.36 t/ha, then the value of this index in case of the variant Geramid New 1.5 l/ha was 45.88 t/ha. With increasing treatment rate, the value of the index in the study decreases and in the case of Geramid New 2.0 l/ha it was 41.32 t/ha (Table 5).

If, in the case of the variants treated with the growth regulator Geramid New, the yield was 41.32-49.36 t/ha, then the Dirager product had a stronger reducing effect on the fruiting organs in the Idared apple plantation, constituting 39.31-46.50 t/ha. Higher fruit yields per unit area were recorded in the Dirager 0.2 l/ha (46.50 t/ha) variant, then in decreasing position the Dirager 0.3 l/ha (43.05 t/ha) variant, the last position going to the Dirager 0.4 l/ha (39.3 t/ha) (Table 5) variant, where the degree of thinning was quite increased. The influence of the Dirager growth regulator, i.e. when the central fruit reached 8-9 mm in diameter, had such a clear influence on the index under study. Treating Idared trees when the central fruit reaches 10-12 mm in diameter will reduce the negative influence on fruit production per unit area.

The Gerba 4 LG product influenced the fruit yield on the studied variants, recording the lowest values of the index taken in the study. So, the average fruit production in the years 2014-2017 for Idared trees in the variant Gerba 4 LG in the dose of 2.0 l/ha was 44.92 t/ha, then the value of this index in the variant Gerba 4 LG in the dose of 2.5 l/ha was 35.74 t/ha, or a decrease by 20.4% compared to the previous variant. The above-mentioned logic is also valid for the variant treated with the growth regulator Gerba 4 LG at the dose of 3.0 l/ha where the fruit yield per unit area decreased by 26.9% compared to the variant Gerba 4 LG 2.0 l/ha and by 8.1% compared to the variant Gerba 4 LG 2.5 l/ha (Table 5).

Table 5. Influence of growth regulators and treatment dose on fruit yield in Idared apple orchard, t/ha

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without thinning (control)	-	51.95	39.28	58.66	38.23	47.03
Hand thinning	-	45.24	45.47	46.26	45.04	45.50
Geramid New	1.2	47.95	50.40	51.90	47.21	49.36
	1.5	45.64	47.21	50.00	40.69	45.88
	2.0	43.80	41.31	41.83	38.33	41.32
Dirager	0.2	46.57	45.98	46.73	46.73	46.50
	0.3	44.78	42.28	46.07	39.07	43.05
	0.4	38.28	39.42	40.40	39.14	39.31
Gerba 4 LG	2.0	44.42	45.78	48.04	41.45	44.92
	2.5	37.33	39.21	29.54	36.90	35.74
	3.0	29.61	35.83	37.78	28.19	32.85
Average	-	43.23	42.92	45.20	40.09	-

The higher temperature of 25ºC during 4-5 days after treatment in the daytime period influenced the degree of reproductive organ thinning in Idared, which is considered to be an easily thinned variety.

The analysis of the experimental data allows us to highlight that the highest production at chemical thinning of the reproductive organs was recorded in the Geramid New variant at the doses of 1.2 and 1.5 l/ha, in the case of the Dirager product variant at the dose of 0.2 l/ha and at the application of the growth regulator Gerba 4 LG in the variant treated with 2.0 l/ha.

The quality of the fruit is a particularly important feature when high yields are recorded per unit area and is characterized by various morphological, organoleptic, technological, biochemical, and finally food value characteristics.

Analysing the fruit diameter, which is a criterion for classifying the fruit into categories during the research and the variants taken in the study (Table 6), we can see that the index taken in the study is influenced by the method of reducing the fruit load, the products used for chemical thinning and the dose administered per unit area.

Lower values of fruit diameter during 2014-2017 in the investigated variants were in the year with higher yields, i.e. in 2016, constituting 77.6 mm.

The highest average fruit diameter was obtained in the year 2017 (81.8 mm), while in the years 2014 and 2015 the studied index recorded average values, constituted 78.5 and 79.9 mm, respectively. These average values over the years of study highlight a large diameter of fruit, which in the end may have a more restricted access to consumers due to the large weight and more difficult preservation in the post-harvest period.

The method of reducing the fruit load also influences the average diameter of a fruit. Lower values of the given index are recorded in the control variant without thinning (72.8 mm) and the variant treated with the growth regulator Geramid New 1.2 l/ha (72.7 mm). Studying how this index developed during the years of research, a more rational correlation of the average diameter of a fruit was scored in the variant Geramid New 1.2 l/ha compared to the control (without thinning). In the control variant due to the large gap per year of fruit production, the index under study was directly influenced. In years with high production (2015; 2017) we record a small average fruit diameter (59.1; 64.3 mm), and vice versa, in years with low production per unit area we record high values, the index under study constituting 81.3 and 86.6 mm, respectively.

The variants treated with Geramid New at the 1.5 l/ha dose was slightly higher (77.2 mm) than the value recorded in the control variant (75.8 mm). The treatment with Dirager at the 0.2 l/ha rate recorded identical values (75.9 mm) to the control variant. The growth regulator Gerba 4 LG which is characterized by a higher degree of thinning of the reproductive organs induced an increase in the index in the study.

Table 6. Influence of growth regulators and treatment dose on quality expressed as mean fruit diameter in Idared, mm

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without thinning (control)	-	64.3	81.3	59.1	86.6	72.8
Hand thinning	-	76.5	75.8	74.5	76.5	75.8
Geramid New	1.2	72.8	71.4	70.7	75.9	72.7
	1.5	77.1	74.7	76.2	80.7	77.2
	20	78.9	80.7	85.7	84.4	82.4
Dirager	0.2	76.8	77.2	71.8	77.7	75.9
	0.3	77.9	78.3	77.2	82.8	79.0
	0.4	86.2	85.7	85.3	82.1	84.8
Gerba 4 LG	2.0	78.2	77.9	78.4	79.0	78.4
	2.5	87.3	87.8	87.5	86.2	87.2
	3.0	88.0	87.7	87.2	88.1	87.8
Average	-	78.5	79.9	77.6	81.8	-

The average diameter of a fruit also changes under the influence of the dose of product applied per unit area. In the variants where growth regulators were studied, we record, that increasing the treatment dose increases the average diameter of a fruit. If, for example, in the variant Geramid New 1.2 l/ha the average diameter of a fruit was 72.5 mm, then with increasing the treatment dose to 1.5 l/ha it was 77.2 mm, and in the variant Geramid New 2.0 l/ha - 82.4 mm. The legality mentioned before is also valid for the growth regulators Dirager and Gerba 4 LG, except that the values taken in the study were higher than in the previous variant, constituting 75.9; 79.0; 84.8 mm and 78.4; 87.2 and 87.8 mm, respectively.

In general, we record that the number of fruits per tree, the average fruit weight, the fruit yield per tree and per unit area, and the average diameter of a fruit correlates with the method of reducing the fruit load, the fruit regulator used for thinning the reproductive organs and the dose applied, as well as the weather conditions during the treatment period and the following 4-5 days.

CONCLUSIONS

Growth regulators based on NAD, ANA and BA in various doses influence differently the number of fruits, their average weight, fruit diameter and the yield.

For a more effective reduction of the fruit load in Idared by chemical method and to exclude the influence of climatic phenomenon it is necessary to have 1-2 growth regulators to be used depending on the respective phenotype.

For the Idared variety higher yields and higher quality were recorded when treating with Geramid New at the rate of 1.2 l/ha at the fall of approx. 80% of flower petals plus 2-3 days. If the weather conditions are not favourable for Geramid New treatment during this period, the growth regulator Dirager can be applied later at a dose of 0.2 l/ha, when the size of the central fruit in diameter will be 8-9 mm. In exceptional cases, when the number of reproductive organs in the crown of the trees is large, should be used Gerba 4 LG at a dose of 2.0 l/ha, when the size of the central fruit in diameter will be 10-12 mm.

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RESPONSE OF SOME APPLE CULTIVARS TO PROHEXADION-CA COMBINED WITH DIFFERENT FERTILIZATION METHODS IN A SUPERINTENSIVE APPLE ORCHARD

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Abstract

Supplying the trees with the optimal amount of nutrients is very important in order to obtain high quantity and high quality yields every year. Prohexadione-Ca is a dual-acting substance that is both a growth regulator and an activator of the natural defense mechanisms of apple trees against pests and diseases. The influence of this compound combined with radicular or foliar fertilizers was evaluated in three apple cultivars: 'Idared', 'Generos' and 'Florina' grafted on medium vigour M26 rootstock. The applied technology in the experimental plot included chemical treatments against the main pests and diseases which commonly affect apple orchards in Northern Transylvania. Different fertilization methods combined with this growth regulator led to a reduction of length of the terminal shoots with direct influence on labour costs and efficiency of orchard management. The results obtained during 2019-2022 revealed the benefits of supplying apple trees with foliar and soil fertilizers for the cultivars to maintain a high productive potential.

***Key words**: apple, fertilizer, growth regulator, terminal shoots*

INTRODUCTION

Apple production represents an important economic activity in Northern Transylvania, Bistrița region. The fertilization of apple orchards with an optimal supply of nutrients in combination with growth regulators, are necessary and very important activities that are required to do in modern fruit trees technology.
Managing the vegetative growth in apple orchards associated with manual pruning is an intensive labour practice, essential to optimize fruit quality and productivity of the trees (Greene, 2007; Cline et al., 2016).
Many methods of growth control have been practiced in the past (Greene, 2003; Miller, 1988), one of the most popular methods is the use of dwarfing rootstocks (Greene, 2007). Uncontrolled vegetative growth affects fruit quality, productivity, pests and diseases control and extremely high pruning costs (Atay & Koyuncu, 2017).
The climate changes in the last years in Bistrița region is causing the modification of flight span and the attack degree of the most damaging apple pests, which can affect the resistance of the trees (Roşu-Mareş et al., 2020).
Prohexadione-calcium (Pro-Ca) is a plant bioregulator (PBR) that was developed by BASF, Germany and Kumiai Chemical Industry, Japan (Meland & Kaiser, 2016). It has been used as an inhibitor of excessive vegetative growth in apples (Privé et al., 2002; 2006), pears (Rademacher, 2016), strawberries (Reekie et al, 2002), and turf (Waddington et al., 1992). It was effective at reducing the incidence of scab (*Venturia inaequalis*) (Spinelli et al., 2010), blossom fire blight and shoot fire blight (*Erwinia amylovora*) (Rademacher et al., 1999; Yoder et al., 1999; Wallis & Cox., 2019) but also can reduce the occurrence of bitter pit (BP) (Amarante et al., 2020). Prohexadione-Ca induces changes in the metabolism of the plants by inhibiting the phenylpropanoids, that have often been found to be involved in defense mechanisms of higher plants (Rademacher et al., 1999) and prevention of the oxidation (Amarante et al., 2019).
Several studies have demonstrated that the prohexadione-calcium (Pro-Ca) has provided for regulating apple tree extension growth ranging from 20 to 60% (Greene, 1996; Byers & Yoder, 1999; Unrath, 1999; Yoder et al., 1999; Basak, 2004; Byers et al., 2004a; 2004b;

Porebski et al., 2006; Privé et al., 2004; 2006; Ramirez et al., 2006; Cline et al., 2008). The applications of Pro-Ca are recommended to be made before and/or after the petal fall phenophase (Rademacher & Kober, 2003). The aim of this research was to evaluate the influence of the growth regulator Regalis Plus combined with different fertilizers on the development and fructification of the apple trees in Northern Transylvania.

MATERIALS AND METHODS

The experiment was carried out in an apple orchard at Fruit Research and Development Station Bistrita (FRDS). FRDS Bistrita is located at 47°10' North latitude and 24°30' east longitude, at 358 m altitude with an average annual temperature around 10°C and multiannual average of 758.80 mm of rainfall, according to the data recorded by the meteorological station at FRDS Bistria, in the last 30 years. The climate is temperate-continental, with relatively hot summers, and less dry cold winters.

The orchard was established in 2001 (22 years old), with 'Idared', 'Generos' and 'Florina' apple trees, on M26 rootstock. The trees are conducted as spindle-bush canopy, 1.5 m between trees and 4 m between rows, with a density of 1667 trees/ha. The experimental design was arranged in 4 replicates for each treatment using 10 trees per replication.

The treatments were applied annually during 2019-2022 growing season. The treatments variants included: V1- fertilization with manure + foliar fertilizer (Cropmax) + growth regulator (Regalis Plus); V2- fertilization with chemical fertilizers NPK+ foliar fertilizer (Cropmax) + growth regulator (Regalis Plus), V3- fertilization with chemical fertilizers NPK, V4- control trees. Regalis Plus was used as a source of Pro-Ca water-dispersible granules. It was applied at a rate of 1.5 kg/ha immediately after flowering and the second applications at a rate of 1.5 kg/ha was made after petal fall when new shoots were less than 10 cm long. Foliar fertilizer Cropmax was applied in a dosage of 2 mL/L water (2 L/ha) four times during the growth season, in four different phenophases: pink buds stage (BBCH 57), fruit size up to 10 mm (BBCH71), fruit size up to 40 mm (BBCH 74) and fruits about half final size (BBCH 75). The soil properties were improved through the application of manure in a dose of 20 kg/tree (33 t/ha) and chemical fertilizers NPK 16:16:16 in a dose of 0.250 kg/tree (416 kg/ha). The assessment of the nutrition supply status of the trees was carried out through soil and foliar diagnosis to determine the quantity in the main chemical elements necessary for a good development of the trees and quality production. The methods used to determine the macro and microelements in the leaves were performed by: Kjeldahl method (dosing by titration) for N total, P (phosphorus) by colorimetric dosing with ammonium metavanadate, K and Ca (potassium, calcium) by flame photometric dosage, Mn, Zn, Cu, Fe, Mg - dosage by atomic absorption spectrometry. Soil analysis were performed by potentiometric, colorimetric, flame photometric and titrimetric, Kjeldahl and Walkley-Black methods. For an efficient pest monitoring, pheromonal traps were installed in each experimental variant. The chemical plant protection treatments included eleven sprays per year. The moment of spray was decided depending on climate conditions and observations of the pests and diseases evolution in the experimental plots. All variants were treated evenly. Products based on mineral oil and copper were used for winter sprays. We used fungicides based on fluxapiroxade, ditianon, difenoconazole, myclobutanil, piraclostrobin, boscalid, folpet, mancozeb, captan, sulfur and tebuconazole against apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), the two main diseases that cause damage in our region. The pests that cause significant damage in Bistrita region are *Cydia pomonella, Anthonomus pomorum, Quadraspidiotus perniciosus, Eriosoma lanigerum, Aphis* spp.*, Panonicus ulmi* and *Tetranichus urticae.* We used insecticides from different chemical groups as follows: acetamiprid, dimetoat, tiacloprid, deltametrin, clorpiriphos methyl, lambda-cyhalotrine, spirotetramate, spinosad; as well as acaricides to control mites: hexitiazox and spirodiclophen.

The evaluations included number and mean length of shoots, fruit yield per tree, mean fruit weight, trunk cross-sectional area and fruit quality. The annual shoots were evaluated each

year with a regular measuring tape, on each tree selected, and compared with the untreated control. Ten vigorous shoots from ten trees were measured at the end of growth period (September) and a visual estimation of the tree vigour was made at the end of vegetation period, when all the leaves had fallen.
Statistical comparisons of the mean values were performed using ANOVA analysis of variance, followed by pairwise correlations with Duncan`s multiple range test with P< 0.0001. aimed by XLSTAT (Addinsoft, France) statistical software package using MS Excel platform.

RESULTS AND DISCUSSIONS

In terms of climatic conditions of Bistrița area, the temperatures recorded in the studied years fluctuated. Annual average temperatures were between 10.5°C (2019) to 11.2°C (2022), with absolute maximum temperatures between 34.6°C (2019) ranging 35.6°C (2022) and absolute minimum temperatures registered in January of each year, recorded values from -11.8°C (2019) to 17.5°C (2022). Regarding the rainfall, the total was 538.9 mm in 2019, 678.9 mm in 2020, 784.5 mm in 2021 and 759.3 mm in 2022 (Table 1). The normal value of annual rainfall in Bistrita region is about 758.8 mm, but the years 1999 and 2000 were very dry compared with 2021-2022. The distribution of rainfall was unevenly that caused an imbalance of growth and fructification processes for apple trees. The difference between 2019 and 2022 was 174.3 mm of annual rainfall.

Table 1. Climate indicators 2019-2020

Climate indicators	2019	2020	2021	2022
Average annual temp. (°C)	10.5	10.1	9.5	11.2
Absolute maximum temp.(°C)	34.6	33.7	33.6	35.6
Rainfall (mm)	538.9	678.9	784.5	759.3

In May 2019 occurred a hail fall of low intensity, which affected part of the apple plots in the FRDS Bistrita area, leading to partial destruction of the foliage of the trees with repercussions on the formation and development of the fruits (Figure 1). The late spring frosts occurred in 4th of April 2020 and 2022 (-5.8°C, respectively -2.2°C) and in 8th of April 2021 (-3.2°C) which did not affect the flower buds, because the values were below their resistance threshold. After analyzing the climatic data, we observed a warming of the weather and the decrease of the rainfall volume in the first two years and climatic accidents (hail and late frosts), which lead to a decrease of the resistance of fruit trees to the attack of diseases and pests, as well as the modification of the manifestation of pathogen infections on fruits and leaves even at varieties with genetic resistance.

Figure 1. The effects of hail fall on foliage and apples

The results regarding the nutrition status of trees indicated an adequate amount of macro and microelements in the soil and leaves (Tables 2 and 3).

Table 2. Soil chemical content

	Deepness cm	Ph	Humus %	Nt %	P mg/kg	K mg/kg
V1	0-20	5.80	4.12	0.241	61.50	356.00
	20-40	5.51	2.83	0.194	18.25	171.25
V2	0-20	5.63	4.05	0.257	65.50	258.75
	20-40	5.48	2.69	0.159	19.75	116.00
V3	0-20	5.70	3.93	0.240	54.50	246.00
	20-40	5.41	3.10	0.175	16.50	139.5
Untreat	0-20	6.02	2.21	0.144	51.00	240.00
	20-40	5.48	3.46	0.191	22.00	173.00

Table 3. Foliar diagnosis

	Macroelements (%)				
	N	P	K	Ca	Mg
V1	2.07	0.12	1.76	0.75	0.23
	2.14	0.22	2.02	1.06	0.29
V2	1.92	0.13	1.66-	1.06	0.27
	2.07	0.20	2.14	1.13	0.31
V3	1.93	0.13	1.68	0.96	0.25
	2.17	0.21	2.01	1.33	0.34
Untrea ted	1.90	0.12	1.56	0.88	0.23
	2.14	0.20	1.67	1.06	0.30

The applied phytosanitary treatments contri-buted to the prevention and combat of the disease and pests flight were monitored with pheromone traps (Figure 2). Considering that all cultivars and all variants were treated with the same treatment scheme, certain cultivars presented a sensitivity to the disease attack.

Figure 2. Pheromonal traps for pests

The number and size of the annual shoots was visibly lower in the treated variant compared to the untreated variant (Figure 3) with the purpose to obtain quality fruit production. Results regarding the number of shoots in the treated variant were between 15-25 shoots/tree when compared with the untreated variant which were between 40-55 shoots/tree.

Figure 3. Untreated variant vs. treated variant

Average length of shoots in the untreated variant had values between 80-87 cm long, and in the treated variant had values between 55-65 cm long (Figure 4).

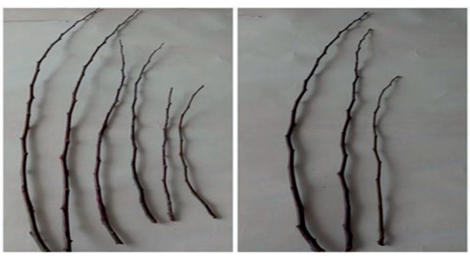

Figure 4. Comparison of shoots length (left-untreated variant, right-treated variant)

After analyzing the data, results showed that the length of annual shoots in all 4 years of study were significantly different in the untreated variant compared with treated variant (V1 and V2) but no significant differences were observed in V3 variant (NPK fertilizer applied but without growth regulator) (Table 4). The time allocated for pruning in the treated variant was significantly reduced due to the reduction of the number and size of the annual shoots.

Table 4. Length of annual shoots (cm)

Variant	2019	2020	2021	2022
Untreated-Florina	83.793 a	83.069 a	86.483 a	87.138 a
Untreated-Idared	80.379 a	79.586 ab	86.448 a	85.310 ab
Untreated-Generos	80.345 a	77.966 ab	85.414 a	84.207 ab
V3 Generos	78.367 ab	72.967 bcd	73.200 bc	74.633 cd
V3 Florina	72.867 abc	68.333 cde	76.633 b	77.567 bc
V3 Idared	67.600 bcd	76.267 abc	73.100 bc	78.933 bc
V2 Florina	61.100 cd	65.633 def	67.367 bc	67.200 de
V2 Idared	58.833 d	67.800 cde	68.767 bc	65.567 e
V2 Generos	57.833 d	67.033 de	67.467 bc	68.900 de
V1 Florina	61.967 cd	68.333 cde	70.467 bc	71.367 cde
V1 Idared	57.267 d	57.833 f	66.067 c	64.833 e
V1 Generos	55.533 d	59.867 ef	68.500 bc	64.633 e

Regarding the influence of Pro-Ca or fertilizers on tree trunk growth over the years, we can conclude that there are slight differences between V1 variants when compared with the untreated variants and chemical fertilizer variants but these results must be correlated also with the synergistic effect of the vigour of rootstock/scion combination, knowing the fact that ‘Florina’ cultivar has a stronger vigour than ‘Generos’ cultivar. The numerical differences between V1 variants and the untreated variants are multifactorial, mainly due to rootstock and scion influence and just a slight effect being produced by Pro-Ca. Analyzed generally, TCSA relatively increased year to year in all variants (Table 5) showing the viability of the scion/rootstock combinations still after 22 years from the establishment of the orchard.
The results regarding the biometric measurements showed that the size and diameter of fruits was specific to the varieties, with the largest fruit in ‘Generos’ cultivar, followed by ‘Florina’ and ‘Idared’.

Table 5. TCSA (trunk cross sectional area cm^2)

Variant	2019	2020	2021	2022
Untreated Florina	142.655 a	155.085 a	158.582 a	161.080 a
Untreated Idared	93.774 e	100.522 d	108.697 d	120.527 c
Untreated Generos	109.753 bcde	117.598 bcd	125.370 bcd	131.353abc
V3 Generos	113.687 bcde	123.364 bcd	130.340 bcd	143.706abc
V3 Florina	129.091 ab	133.279 ab	137.006 abc	148.025 ab
V3 Idared	115.955 bcde	128.253 abc	138.533 ab	148.025 ab
V2 Florina	121.242 abcd	128.091 abc	134.414 abcd	139.348abc
V2 Idared	94.986 de	105.819 cd	117.085 bcd	127.364 bc
V2 Generos	106.443 bcde	116.000 bcd	123.888 bcd	133.132abc
V1 Florina	123.413 abc	130.224 ab	137.006 abc	141.702abc
V1 Idared	93.878 e	105.819 cd	109.269 cd	120.527 c
V1 Generos	101.570 cde	109.470 bcd	118.001 bcd	125.099 bc

The weight of the fruits varied between 147.7-262.7 g for 'Generos', for the 'Florina' the weight of the fruits ranged between 131.8-223.8 g and 125.3-203.6 g for the 'Idared' cultivar. The Pro-Ca did not have a significant effect on fruit development, weight or quality. The fruits were at optimal level of quality for 'Generos' and 'Florina' cultivars, exception made by 'Idared' cultivar, where the fruits showed a higher sensitivity to the attack of diseases on the fruits. The statistical data indicated that the V1 'Florina' variant was statistically different than the other V2 and V3 variants, showing the great influence of the applied manure together with the foliar fertilization completed with the productiveness and vigour of the 'Florina' cultivar, being a multifactorial influence of variant V1. On the other hand the other V1, V2, V3 variants were statistically not different among them being in the same statistical class when compared with the untreated 'Idared' cultivar (Table 6).

Table 6. Fruit production

Variant	Fruit Production (kg/Tree)	Yield (tons/ha)
V1 Florina	31.025 a	51.700 a
V2 Florina	26.250 ab	43.738 ab
V2 Generos	25.720 ab	43.485 ab
V1 Generos	25.325 ab	42.193 ab
V1 Idared	22.850 ab	38.055 ab
V3 Generos	22.750 ab	37.750 ab
V3 Florina	22.748 ab	37.350 ab
V2 Idared	21.425 ab	37.000 ab
Untreated Generos	16.500 ab	27.805 ab
V3 Idared	16.200 ab	26.233 ab
Untreated Florina	15.690 ab	25.975 ab
Untreated Idared	14.675 b	20.350 b

The untreated 'Idared' variant in our research produced the lowest yield per tree and per hectare. The registered values were between 14.67-16.5 kg/tree for control variant (without soil or foliar supply) and 21.42-31.025 kg/tree for treated variants.

CONCLUSIONS

In all three cultivars 'Generos','Idared' and 'Florina' yield and average fruit weight at harvest stage was not significantly different among treatments, while the influence of growth regulator Regalis Plus on the reduction of annual growth was significantly different, compared with the control. The time allocated for pruning in the treated variant was significantly reduced due to the reduction of the number and size of the annual shoots, with direct influence on labour costs and efficiency of orchard management.

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THE INFLUENCE OF THE TYPE OF CUTTING IN THE RASPBERRY SPECIES ON THE QUALITY OF THE PRODUCTION OBTAINED

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Abstract

Even though raspberry cuts are simple, opinions differ when it comes to raspberry cuts. In general, for the exercise of a modern orchard, the varieties must be adapted to modern mechanization and soil maintenance technologies. Following the research carried out, we can specify the fact that the Cayuga variety has a high drajon capacity, and a greater shortening of the stems leads to a decrease in drajon production. In the variants where the inflorescence's were not removed, the production of raspberry stems does not decrease. The quality of the raspberry stems was influenced to a very small extent by the removal or not of the inflorescence's. The root system of the raspberry is more superficial, the vast majority of the roots, about 80% of the roots are located closer to the soil surface, respectively in the soil layer between 0-20 cm. An intense growth of raspberry stems takes place in the months of May-June and the beginning of July, but along the way the pace of growth slows down, thus at the end of August their growth stops.

Key words: *raspberry species, variety, shortening, quality.*

INTRODUCTION

In order to obtain large and quality productions, it is necessary to find methods of obtaining an increasingly better planting material, which corresponds both in terms of production quality and quantity. A major interest is to maintain the genetic material at a real biological potential and at the same time to apply specific culture technology (Butac, 2008). Many researches have been carried out in the country to improve the characteristics of the varieties of fruit trees in order to increase the production and quality of the fruits.

The characteristics of some raspberry varieties created in Romania between 1967 and 2007, namely varieties such as Citria, Ruvi, Star, Opal and Gustar, were presented respectively aspects of the fruit, of the plant, aspects of the production and use of the fruit., (Braniște et al., 2007). Within the Pitesti-Mărăcineni Research Institute, the foundations were laid for obtaining new varieties with greater adaptability to climatic conditions and greater tolerance to diseases and pests, thus in this sense new raspberry varieties were obtained such as be Star, Ruvi and Citria (Mladin, 2002). Research on raspberry culture has expanded in our country, thus establishing the most suitable varieties for the southern part of the country, especially for the psammosol area (Popescu et al., 1989). In the lowland areas, on the basis of the researches, it was reached the expansion of raspberry varieties resistant to frost and drought, such as Cayuga, Romy, (Botez et al., 1984). Also, based on the research carried out, it was found that the R 3-3-30 and R 5-3-30 FC selections have large, firm fruits and a good winter resistance (Mladin et al., 2008). Some research carried out on raspberry varieties in Estonia shows that some new raspberry varieties Aita and Alvi are promising due to their large fruits and very good resistance to anthracnose. Helkal and Tomo varieties had the highest sugar content (Arus et al., 2008). In the northern area of Montenegro, research was carried out on the propagation of raspberries, to improve the characteristics of the fruits and to increase the resistance to different biotic and abiotic factors (Galic et al., 2012). Raspberry growth can be affected by some plant materials used as mulch, thus mulching with wheat straw can affect plant growth, (Pedreros et al., 2008). The optimization of production systems can be achieved on organic matter substrates such as wood bark, bark compost, wood fibers (Carlen et al., 2020). The examination of the growth mode of seven Primocane raspberry cultivars in order to observe whether these cultivars are suitable for the production of a summer crop, states that of the studied cultivars only Imara and Kwanza are suitable for the production of a summer crop (Palonen et al. , 2020). In Latvia, some raspberry varieties such as Gerakl, proved good adaptability to the respective region and the variety Babye Lato 2 recorded the highest productions (Laugale, 2012). The history of raspberry and blackberry cultivation in the southern USA states that 20 cultivars, including 12 cultivars with interspecific hybridization, have been created in different states of Arkansas, Florida, Virginia (Ballinghton, 2016). Fertilization with different macronutrients in raspberries with N, P, K, Ca and Mg, shows some obvious toxicity symptoms with N and K in Dormanred cultivar (Spires et al., 1999). The behavior of three raspberry cultivars under modified atmosphere conditions was studied, where Qualicum and Chilliwack cultivars showed better firmness and better keeping characteristics (Toivonen et al., 1999). The studies on the temporal evolution of some parameters in the current climate over Romania have highlighted a trend of increasing air temperature and related parameters, an aspect that can bring changes in the growth of fruit trees and shrubs (Velea et al., 2021). Worldwide, pathogens represent one of the most important biotic stress factors of fruit trees and scrubs, limiting their proper growth and affecting their genetic reproduction capacity and yielding potential, leading sometimes even to the whole tree dieback (Cotuna et al., 2020). Observations on the factors that influenced the growth and some phenological phases of several raspberry varieties in Moldova (flowering period, etc.), indicate that the Cayuga variety is one of the varieties resistant to low temperatures in winter (Sava, 2013). Norwegian raspberry cultivars tested in an infested field for root rot revealed that most cultivars were infested, except Varnes (Roslash et al., 2002). An evaluation of the vegetative growth of some raspberry and blackberry cultivars was carried out in southern Poland, where it was stated that the Willamette variety had the highest number of stems but also the highest number of leaves per stem, and the Polesie variety had obtained the largest fruits (Orzel et al., 2016).

MATERIALS AND METHODS

The biological material is represented by the Cayuga raspberry variety, the planting distance being 2.5 m between rows and 1.0 m between plants per row, respectively 4000 plants per hectare. The experience took place in 2019, the studies taking place between 2019 and 2021. The experience includes 9 variants in 4 randomized repetitions, namely: V1 - Cut to 20 cm with the removal of the inflorescence (Control); V2 - Uncut and leave the inflorescences; V3 - Uncut and inflorescences removed; V4 - Cut to 100 cm and leave the inflorescences; V5 - Cut to 100 cm and remove inflorescences; V6 - Cut to 50 cm and leave the inflorescences; V7 - Cut to 50 cm and remove inflorescences; V8 - Cut to 20 cm and leave the inflorescences; V9 - Removed the aerial part entirely. The experience was located in the town of Breasta, Dolj county, on a land sheltered from the prevailing winds with protective curtains. The objective of the work is the following:

- studying the influence of the shortening of raspberry stems on the quantity and quality of the production of planting material in order to establish the optimal shortening of the stems;
- if the removal of inflorescences influences the quantity and quality of planting material. In order to achieve the established objectives, the following observations and determinations were made: the production of suckers; the dynamics of suckers growth; quality of suckers (height and thickness); the distribution depth of the horizontal roots by the profile method; the quality of the fruits, respectively some physical and chemical properties of the fruits.

RESULTS AND DISCUSSIONS

The growth characteristics of raspberries state that raspberry suckers live independently after forming their own roots and becoming fruiting stems, (Chira, 2000). Raspberry suckers begin to appear in April and to study the dynamics of their growth the first measurement was carried out on May 13. The next three measurements were made at an interval of 14 days, after which they were made every 7 days until the cessation of suckers growth was observed. Looking at the dynamics of the increase in length of suckers depending on the applied treatment, we find that at the time of the first measurement (May 13), the average length of suckers was close in most variants. It can be seen that this growth of suckers is very intense in the months of May - June and the beginning of July, registering large increments in the length of suckers.

For example, in the V1 variant (cut to 20 cm and removed the inflorescences), the increase in growth was 70 cm from May 13 to June 25 and 37 cm from June 25 to July 30. The lowest growth rate during this period can be observed in the V8 variant (Cut to 20 cm and leave the inflorescences) and in the V9 variant (removed the aerial part), where the growth increments were 18 cm in the V8 variant and 16 cm in the V9 variant between May 13 and July 30. Analyzing the data further, we can see a decrease in the rate of growth for all variants between June 25 and July 30, thus variant V 6 recorded an increase of 36 cm, V1 of 33 cm, V 7 of 31 cm, V 5 of 25 cm, V 2 of 23 cm, V 4 of 22 cm. Thus, the increase in length from June 25 to July 30 is between 16 cm for the V9 variant and 37 cm for the V3 variant (Figure 1).

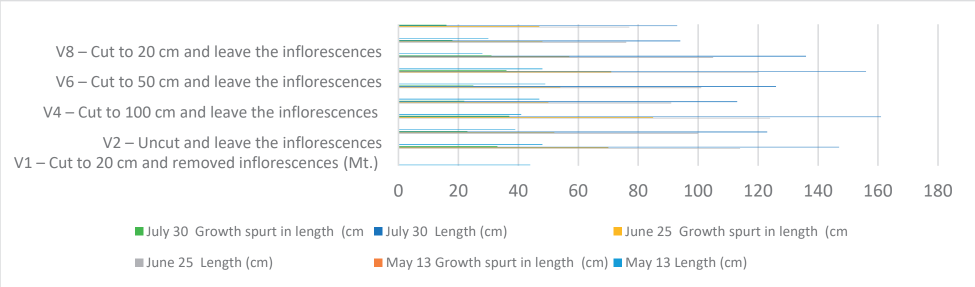

Figure 1. The dynamics of suckers growth in length depending on the applied treatment (period 14 May - 30 July)

Next, the rate of growth in the length of the suckers becomes upward until around August 20, thus the length of the stems fell within the ranges of 191 cm variant V 3 and 93.0 cm variant V 9. An upward growth in addition to the V9 variant was also recorded in the V 6 variant - 182.0 cm, the V 1 variant - 160.0 cm, the V 5 variant - 149.0 cm.

On August 20, the growth spurt was between 30 cm (variant V 3) and 6.0 cm (variant V 2). The V7, V 8 and V 9 variants no longer had an increase in length. After August 20, growth stops due to the fact that part of the nutrients are directed to the fruiting process (Figure 2).

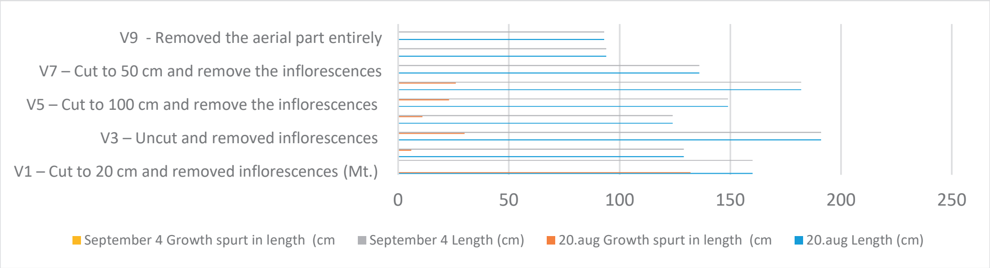

Figure 2. The dynamics of stems growth in length depending on the applied treatment (period 14 May - 04 September)

The greatest increase in length between May 13 and September 4 was recorded for the variants V3 - 152.0 cm, V 6 - 133.0 cm, V 5 – 126.0 cm and V 1 - 116.0 cm. The smallest increase in length was observed in variants V 7, V 8 and V 9 (Figure 3).

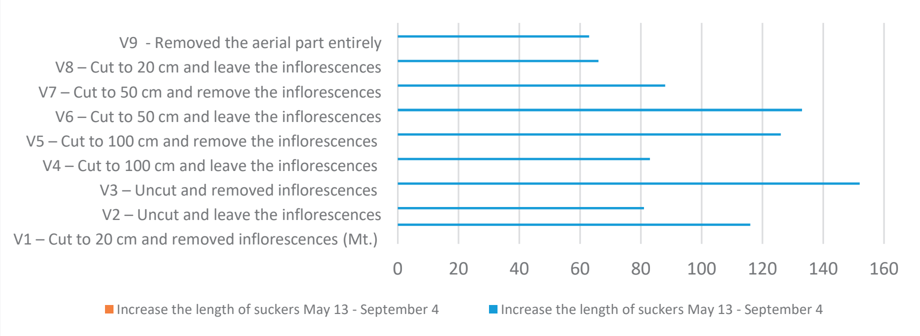

Figure 3. Increase the length of suckers (period May 13 - September 4)

Following the quality of suckers in 2020 (Figure 4), we notice that the average length of suckers falls between 103.0 cm for the V9 variant (removed the aerial part) and 137.0 cm for the V2 variant (uncut and leaving the inflorescences), the values being insignificant. Only for the V9 variant where the aerial part has been removed, the value is negative. The diameter of the suckers was influenced to a very small extent by the applied treatment, the differences being only 0.13-0.77 mm between the variants, but the values being insignificant. The average diameter of the suckers is between 6.5 mm for the V6 version and 7.93 mm for the V5 version, falling within the STAS values.

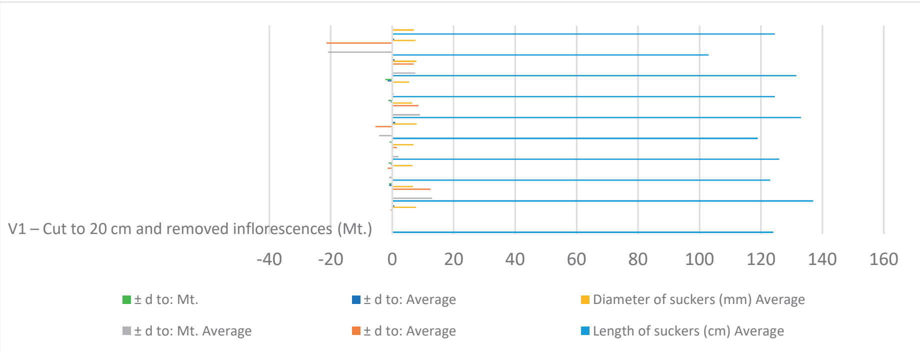

Figure 4. The influence of the degree of shortening and removal of inflorescences on the quality of raspberry suckers

Analyzing the percentage of suckers by length categories according to the degree of shortening and the removal or not of the inflorescences (Figure 5), we notice that the percentage of suckers with a length of less than 50 cm is between 1.9 % for variant 3 and 6.3% for variant 1. The percentage of suckers with lengths of 50-80 cm is between 1.8% for variant 8 and 17.9% for variant 2.

In the 81-110 cm category, the percentage of suckers is 16.7% in variant 1 and 58.0% in variant 9. In the 111-140 cm length category, the percentage of suckers is 28.0% in variants 9 and 2, and 62.3% for option 8. In the category between 141 and 170 cm, the lowest percentage is recorded in variant 9 of 7.1%, and the highest of 30.2% in variant 4. In the category between 171 and 200 cm, the lowest percentage of suckers is 1.8% in option 7, and the highest percentage was recorded in option 3 of 13.0%. In the over 200 cm category, the lowest percentage was recorded for version 2 of 3.0% and the highest for version 3 of 7.0%. From the analysis of suckers by length categories expressed in percentages, we found that most suckers fall between 81 and 160 cm long.

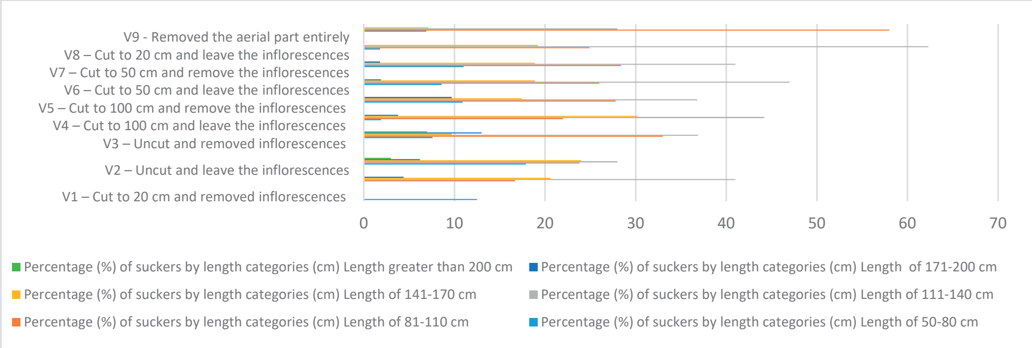

Figure 5. The percentage of suckers by length categories according to the degree of stems shortening and the removal or not of inflorescences

Studying the distribution of horizontal roots by the profile method, depending on the degree of shortening of the suckers and the removal or not of the inflorescences, we noticed that most of the horizontal roots are found in the soil layer from 0-10 cm and 11- 20 cm (Figure 6). Some of the roots reach deeper, but not in too great a number (Table 1 and Table 2).

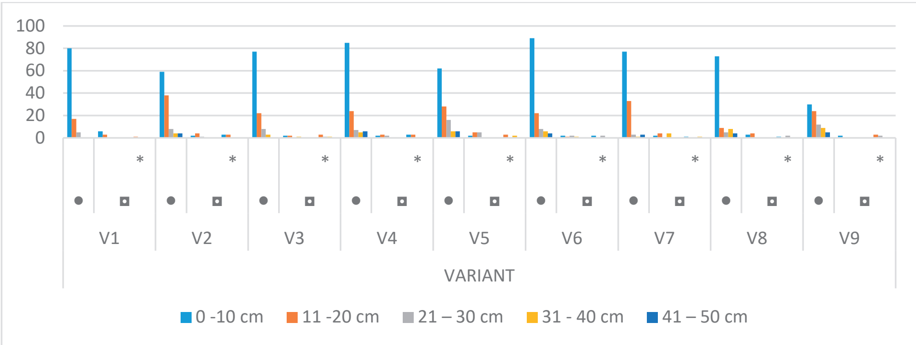

Figure 6. Distribution depth of raspberry roots according to the degree of stem shortening and the removal of inflorescences

Table 1 - Distribution depth of raspberry roots according to the degree of stem shortening and the removal of inflorescences

The distribution depth of the roots	VARIANT														
	V1			V2			V3			V4			V5		
	●	◘	*	●	◘	*	●	◘	*	●	◘	*	●	◘	*
0-10 cm	80	6	-	59	2	3	77	2	-	85	2	3	62	2	-
11-20 cm	17	3	1	38	4	3	22	2	3	24	3	3	28	5	3
21-30 cm	5	-	-	8	1	-	8	-	1	7	2	-	16	5	-
31-40 cm	-	-	-	4	-	-	3	1	1	5	-	-	6	-	2
41-50 cm	-	-	-	4	-	-	-	-	-	6	-	-	6	-	-
Total roots by category	102	9	1	113	7	6	110	5	5	127	7	6	118	12	5
TOTAL ROOTS	112			126			120			140			135		

Table 2 - Distribution depth of raspberry roots according to the degree of stem shortening and the removal of inflorescences

The distribution depth of the roots	V6			V7			V8			V9		
	●	◘	*	●	◘	*	●	◘	*	●	◘	*
0-10 cm	89	2	2	77	2	1	73	3	1	30	2	-
11-20 cm	22	1	-	33	4	-	9	4	-	24	-	3
21-30 cm	8	2	2	3	-	-	5	-	2	12	-	2
31-40 cm	6	1	-	1	4	1	8	-	-	9	-	-
4150 cm	4	-	-	3	-	-	4	-	-	5	-	-
Total roots by category	129	6	4	117	10	2	99	7	3	80	2	5
TOTAL ROOTS	139			129			109			87		

Legend: ● - roots less than 1 mm in diameter; ◘ - roots with a diameter between 1 –2 mm; * - roots over 2 mm in diameter

An essential condition of the functionality of the root system is the exploitation by the root system of a volume of soil as well as the horizontal and deep extension of the roots (Voiculescu et al., 2001). The roots are generally superficial in raspberries and have the particularity of forming buds from which root shoots appear, (Militiu et al., 1962). The roots have the role of ensuring an absorption of nutrients from the soil, which will help an optimal differentiation of the fruit buds (Cichi et al., 2019).

Analyzing the percentage of roots at the depth of 0-50 cm for each variant, the situation is as follows:

Variant 1 (Cut to 20 cm and removed the inflorescences) at the depth of 0-10 cm 76.7% of the total roots were found, at the depth of 11-20 cm 18.7 % were found and only 4.4% up to 30 cm deep (Figure 7).

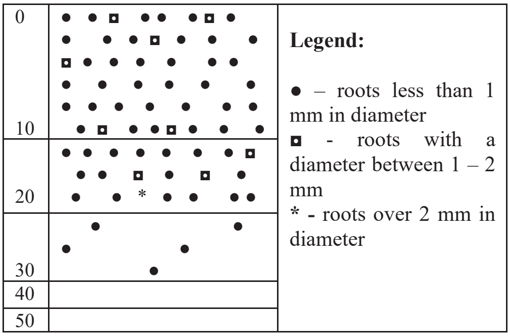

Figure 7. The distribution depth of the roots at V1

In variant 2 (Uncut and leave the inflorescences) in the first layer at the depth of 0-10 cm 50.7% were found, in the second layer of 11-20 cm the percentage was 35.7 %, 21-30 cm 7.1 % were found at 41-50 cm, 3.1% were found at 31-40 cm and 41-50 cm (Figure 8).

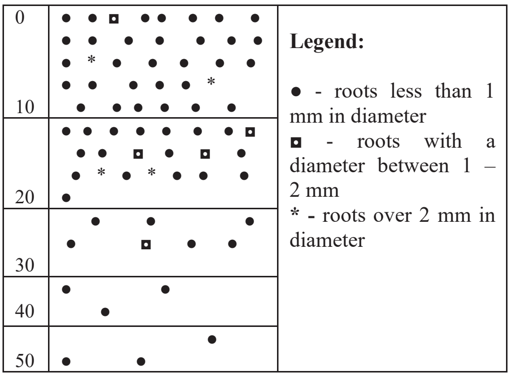

Figure 8. The distribution depth of the roots at V2

In variant 3 (Uncut and inflorescences removed) at the depth of 0-10 cm, 65.8% of the total roots were found, at the depth of 11-20 cm, 22.5% and 7.5 % up to 21-30 cm deep, and in the layer from 31 - 40 cm only 4.1 %, (Figure 9). In variant 4 (Cut at 100 cm and leave the inflorescences) in the first layer of 0-10 cm 64.2 % were found, in the second layer 21.4 %, from 21-30 cm 6.4 %, at 31-40 cm were recorded 3.5% and at 41-50 cm 4.2% were found (Figure 9). In variant 5 (Cut at 100 cm and removed the inflorescences) in the first layer of 0-10 cm 47.4% were found, in the second layer 26.6%, from 21-30 cm 15.5%, at 31-40 cm were recorded 5.9% and at 41-50 cm 4.4% were found (Figure 9).

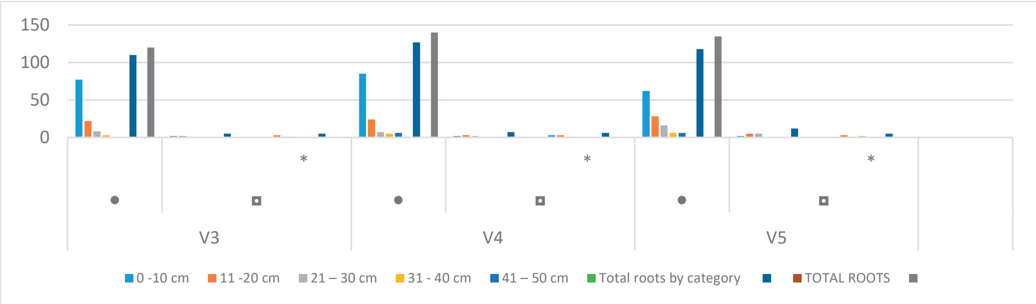

Figure 9. Distribution depth of raspberry roots according
to the degree of stem shortening and the removal of inflorescences

In variant 6 (Cut to 50 cm and leave the inflorescences) in the first layer of 0-10 cm 66.9% were found, in the second layer 16.5%, from 21-30 cm 8.6%, at 31-40 cm were recorded 5.0% and at 41-50 cm 2.8% were found (Figure 10). Next, in variants 7, 8 and 9, the highest percentage of roots is also found in the 0-10 cm layer, respectively V 7 - 62.0%, V 8 - 70.6% and V 9 - 36.7%. At the depth of 11-20 cm the percentage of roots was 28.6% - V 7, 11.9% at V 8, and 31.0% at V 9. Only a percentage of 2.3% - 3.6% - 5.7% is in the layer between 41-50 cm in variants 7, 8 and 9, (Figure 10).

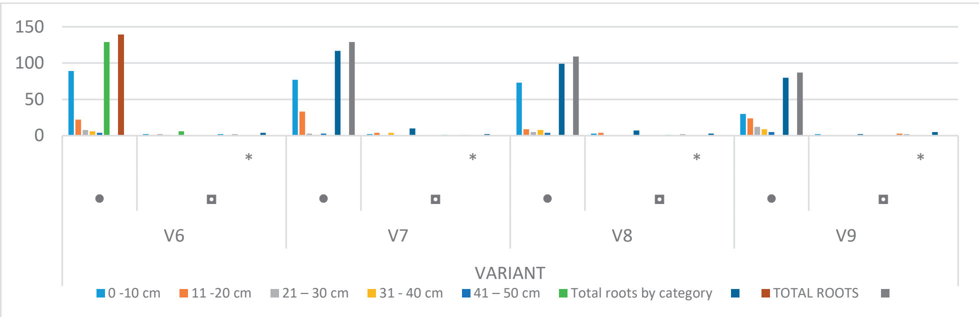

Figure 10. Distribution depth of raspberry roots according
to the degree of stem shortening and the removal of inflorescences

The root is formed by the rhizome (underground stem) from which the vast majority of roots start at a depth of 10-50 cm and very few reach deeper, (Popescu et al., 1992). Knowing the distribution of horizontal roots in the soil helps to distribute fertilizers in the place explored by the largest mass of roots, (Cichi et al., 2008). Due to the fact that raspberry roots develop more on the surface of the soil, it should be pointed out that the soil work should be carried out at a depth of 0-10 cm per row and 5-10 cm between rows. Root system growth occurs throughout the growing season, but the intensity of growth varies. The most intense growth is observed during the flowering period and after harvest. Soil work must be carried out during the period of minimum root growth, that is, before the buds open and in September. The fruits obtained are large, with an average weight of 2.5-2.8 g reaching up to 3.0 g. Regarding the chemical composition, they contain 4.0-4.10 g of sugar per 100 g of fresh pulp, 1.60 g per 100 g fresh pulp acidity expressed in malic acid and 26.10 mg per 100 g fresh pulp ascorbic acid. The Cayuga variety has a soluble dry matter content of 11.2%.

Along with the physical and chemical properties of the fruits, the very pleasant aroma and the possibility of obtaining numerous industrialized products, we appreciate the southern part of the country as a favorable area for this culture. The evaluation of the characteristics of an individual from a certain region has an important role in identifying the agrobiological performances of that individual (Cichi et al., 2022).

CONCLUSIONS

The rooting capacity of the Cayuga variety is generally medium to high. The severe shortening of the stems and the total removal of the aerial part leads to a decrease in the production of suckers. The moderate shortening to about 100 cm with the preservation of the inflorescences on the stems of two years, allow obtaining productions of 100 thousand pieces of suckers/ha and at the same time obtaining very good fruit productions. In the variants where the inflorescences were not removed, the production of suckers does not decrease, on the contrary, the production increases. Raspberry suckers have an intense growth in the months of May-June and early July, then the rate of growth decreases so that at the end of August the growth of suckers stops. The quality of the suckers was influenced to a very small extent by the treatment applied and by the removal or not of the inflorescences, the results obtained were not significant. Most of the suckers obtained have a length between 85-180 cm, respectively in a percentage of 75- 80.0%. The root system of the raspberry is quite superficial, about 60-77.0% of the roots are located in the soil layer between 0-10 cm, in the soil layer between 11-20 cm there is a percentage of 11.0-35.0% roots and only a percentage of 2.3-5.7% reach up to 41-50 cm depth. It is necessary to research different varieties in different areas in the context of climate change.

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EFFECT OF ORGANIC FOLIAR FERTILIZERS ON YIELD AND FRUIT QUALITY OF SEVEN HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) CULTIVARS

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Abstract

Consumer-valued blueberry fruits are increasingly sought after for consumption in fresh or processed form due to their beneficial effect on human health, as supported by extensive scientific evidence. In this study we looked at the effect of organic foliar fertilizers on production and on the quality parameters of fruit in seven blueberry varieties. The experiment was carried out at ICDP Pitești-Mărăcineni, over the course of the years 2020-2022, on a plantation established in 2018, and was presented in a randomized block design with three repetitions and three fertilization variants: control group (untreated), Codamix (0.25%), and Ecoaminoalga (0.25%). The treatment was repeated three times at an interval of 14 days, beginning with the formation of the bud until the start of fruit ripening. At the harvest time, production data and the following quality indicators were measured and recorded: fruit mass, firmness, color, soluble dry matter content, total titratable acidity, and total sugar content. The results showed that organic fertilizers had a positive effect on both the quantity and quality of blueberry fruits produced during this study.

Key words: *blueberry, organic fertilizer, production, fruit quality.*

INTRODUCTION

The blueberry *Vaccinium corymbosum*, originally from North America, a member of the *Ericaceae* family, has become an international crop, currently enjoying wide popularity (Retamales & Hancock, 2012). The interest in cultivating this species is due to its high profitability and special nutritional benefits. According to the opinion of many researchers, the consumption of blueberries contributes to the prevention of many cardiovascular and neurological diseases, cancers, osteoporosis, diabetes, due to the anti-inflammatory and antioxidant effect of the phenolic compounds found at a high level in the fruit (Curtis et al., 2019; Si, 2020). It has been mentioned the improvement of memory and cognitive performance at all ages (Miller et al., 2018; Bell & Williams, 2021), the delay of neurodegenerative disorders and brain aging (Youdim & Joseph, 2001) due to the regular consumption of these fruits. Previous studies have shown that growing blueberries without fertilizer produces almost negligible yields; therefore, fertilization is necessary to ensure adequate productivity (Albert et al., 2011). In recent decades, organic crop production has gained the attention of many farmers (Neilsen et al., 2009). Fruits obtained through organic cropping systems are valued due to their higher nutritional quality (Saba & Messina, 2003). Numerous researches have proven the beneficial effect of organic fertilizers on the physico-chemical and biological properties of soil and crops (Manea et al., 2019, Khan et al., 2020, Wajid et al., 2020). Blueberry fertilization has been the focus of many studies. The blueberry prefers acidic soils, well-drained, moist soils rich in humus and poorer in nutrients compared to other fruit species (Zydlik et al., 2019). The use of organic fertilizers in critical phases of plant development is of particular importance for improving yields (Kandil et al., 2017; Mahmood et al., 2020) and fruit quality (Al-Kharusi et al., 2009; Schoebitz et al., 2019). Fertilizing the plant's roots is the main way of providing the plant with nutrients. Organic fertilizers delivered directly to the soil require a long decomposition time and provide a lasting effect on crops (Sharma & Mittra, 1991). Foliar

practice quickly replenishes the supply of minerals needed by plants (Wach & Błażewicz-Woźniak, 2012; Karlsons & Osvalde, 2019). Substances applied by spraying are absorbed much faster than those applied on the ground (Ciavatta & Benedetti, 2002).
Extraradicular administration of treatments early in the morning or in the evening when the stomata are open is recommended. The temperature of the environment must be a maximum of 27°C. Spray solutions should cover the entire surface of the leaves. For organic cropping systems, recommended products provide a lower input of phosphorus or potassium than chemical ones (Green, 2015). Foliar solutions generally contain free amino acids and peptides. They require a period of 3-7 days to be absorbed (Umemiya & Furuya, 2001). Applied nitrogen can influence fruit weight, soluble solids levels, and the amount of pigments in the fruit epidermis (Jiao et al., 2017). Sugar content can be influenced by culture systems, genetic background, environmental conditions, etc. (Gündoğdu, 2019). It is believed that seaweed extracts can improve the soluble solids content of blueberries (Loyola & Muñoz, 2008). However, Panicker et al. (2016) reported a similar soluble solids content to the untreated variant. In this study, we followed the effect of foliar fertilizers compatible with organic agriculture on the production and on some fruit quality parameters of seven blueberry varieties under Romanian conditions.

MATERIALS AND METHODS

The experiment was carried out at ICDP Pitesti-Mărăcineni, between the years 2020-2022, within the Genetics and Breeding Laboratory on a plantation established in 2018. From a geographical point of view, the Institute is located at 24°55' east longitude and 44°55' north latitude, the altitude of the land being between 200 and 290 m. The experiment was located on a flat, alluvial type, on a brown-clay soil with a clay-clay texture in the first 60-70 cm, and in depth the texture becomes sandy. The plants were placed on drums covered with black polyethylene. The soil was improved by adding acid peat along the plant rows (30 t/ha). The results of the agrochemical analyzes at the end of the fruit harvesting season are presented in Table 1.

Table 1. Soil chemical analyzes in the blueberry plot at fruit harvest(period 2020-2022)

Depth (cm)	pH in water	Acidity hydrolytic (me/100 g soil)	The sum of the bases (me/100 g soil)	Cation exchange capacity T (me/100 g soil)	The degree of saturation with bases V%	Potassium K ppm	Phosphorus P_2O_5 ppm	Total organic carbon %	Humus %	IN	Total nitrogen %
0-10	5.28	5.89	12.74	18.63	68.36	178.80	24.29	7.39	12.74	8.71	0.61
10-20	5.26	5.03	22.76	27.79	81.89	164.25	21.43	3.30	5.68	4.66	0.27
20-30	5.48	3.67	13.56	17.22	78.71	169.71	24.29	2.44	4.20	3.31	0.20
30-40	5.66	3.88	13.39	17.27	77.53	169.71	15.71	2.52	4.34	3.36	0.21

The experiment was carried out in a randomized block design with three repetitions and three variants of extraradicular fertilization: control (untreated), Codamix (0.25%) and Ecoaminoalga (0.25%). Codamix (V2) is a complex of trace elements chelated with citric, lignosulfonic acid, soluble in water specifically to supplement the NPK supply. Ecoaminoalga (V3) has as basic elements amino acids, gibberellins, marine algae and microelements: Mn, Cu, Fe, Zn, B. The treatments were repeated three times at an interval of 14 days during the time from bud formation to at the beginning of fruit ripening. Seven blueberry cultivars were analyzed ('Simultan', 'Safir', 'Delicia', 'Vital', 'Pastel', 'Elliott' and 'Duke'). Production data were recorded and the following quality parameters were determined: fruit weight, firmness, color, soluble dry matter content, total titratable acidity and total sugar content.
To determine the **productivity**, the fruit production on each plant was weighed (in g/plant).
The **fruit weight** was determined according to the ripening period of each variety by weighing all the fruits in a sample (20-25 fruits) and the average weight of the fruits was calculated, in g/fruit, using a Kern balance, precision 0.00.
Fruit firmness was expressed in HPE units and determined with the non-destructive Qualitest HPE penetrometer equipped with a 0.25 cm^2 diameter tip.

Skin color was determined using the Konica Minolta CR 400 colorimeter in the system (L, a*, b*). The CIELAB color space is organized in the form of a cube. The maximum for L* is 100, which represents the color white, and the minimum for L* is zero, which represents black. Axes a* and b* have no specific numerical limits. Positive values for a* show the color red and negative values show the color green. Positive values for b* show the color yellow, and negative values show the color blue.

Soluble solids content, expressed in % Brix, was determined with a digital Hanna refractometer.

Total acidity. The organic acid content of blueberry fruit was determined by the titrimetric method, using 25 ml of aqueous fruit extract neutralized with a 0.1N NaOH solution in the presence of phenolphthalein as an indicator. The total acid content was expressed as malic acid (%).

Total sugar content was estimated by the Fehling-Soxhlet method, 1968 (JAOAC, 1968). The principle of the method is based on the oxidation reaction between the copper in the copper alcoholate of sodium and potassium tartrate and the aldehyde and ketone grouping of the reducing sugars. This method determines the amount of reducing sugar which reduces a certain volume of Fehling's reagent. Total sugar content was expressed as a percentage (%).

All analytical determinations were performed on three replications, and the data were subjected to analysis of variance (ANOVA). The influence of experimental factors was analyzed by the Duncan test, with a significance level of $p \leq 0.05$. Correlations were also made between the biochemical quality indicators of the fruits. Statistical data analysis was performed using SPSS 14.0 for Windows software.

RESULTS AND DISCUSSION

Crop yield was significantly higher in the case of the variant fertilized with Ecoaminoalga - V3 (633.72 g/bush respectively 2.11 t/ha) compared to the untreated control variant - V1 (577.96 g/bush respectively 1.93 t /ha), on the average of the variants achieving an increase of 9.6% compared to the control variant (Figure 1). Koort et al. (2020) stated that organic fertilizers have a significant effect on crop yield, also.

The data of the present study show that the composition of fertilizers is also significant on plant productivity and the response of the variety may be different.

Among the varieties, Delicia stood out with an average production during 2020-2023 of 806.11 g/plant (2.69 t/ha), followed by the Vital varieties with 669.59 g/plant (2.23 t/ha) and Duke with 634.42 g/plant (2.11 t/ha).

During the experimental period it was observed that the Simultan variety had the lowest productivity 470.96 g/bush (1.57 t/ha).

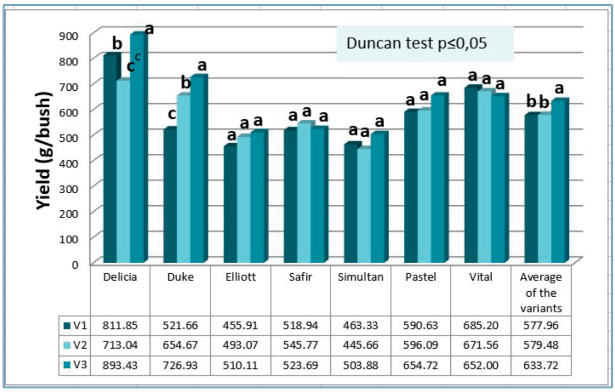


Figure 1. The effect of the fertilization option on blueberry fruit production according to variety in the Argeș area 2020-2022

ANOVA variance analysis data show that fruit production, in g/plant, was significantly influenced by the genetic background of the cultivar with 13.9%, environmental conditions with 24.8% (p=0.000) and the fertilization variant with only 1.6%.

The differences in productivity between years are also due to the fact that the plants were growing and the harvest was just beginning. Once the crop reaches full maturity, yield may fluctuate from year to year due to weather conditions, crop maintenance practices (cutting), etc. (Ranganna, 1986).

Other researchers have mentioned that the production of this crop can be influenced by environmental conditions, agricultural practices, soil conditions (Ranganna, 1986; Schoebitz et al., 2019).

Fruit weight. The weight of the berries, expressed in grams, was higher in the case of variant 3 - Ecoaminoalga (2.15 g) (Figure 2) statistically not differentiated from the non-fertilized variant (2.09 g). In the case of the Codamix fertilizer, this was significantly lower (2.01 g). Differences in fruit weight are observed between varieties. The average upper weight was higher in the varieties Vital (2.33 g) and Simultan (2.32 g). In the Duke variety, the average weight of the fruit was only 1.71 g, being significantly higher in the case of the V3 variant (2.14 g). It can be said that the composition of the applied foliar fertilizer can have significant effects on the mass of berries. Some fertilizers may have a beneficial effect but without an obvious trend. This fact was also noted by Koort et al. (2020).

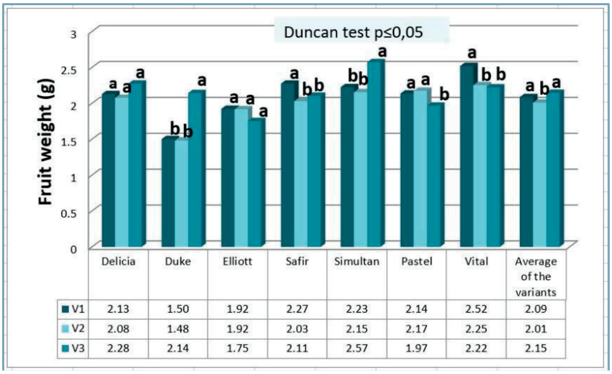


Figure 2. The effect of the fertilization option on the weight of the berries depending on the variety in the Argeș area 2020-2022

Fruit firmness. Evaluation of fruit firmness is important for assessing the quality of fruits in the marketing chain, especially those intended for fresh consumption, in breeding programs and for researchers in variety testing (Sekse et al., 2011). It is considered to be dependent on fruit water level, cell wall metabolism, and fruit cuticle structure (Paniagua et al., 2013). This can decrease as the berries reach the optimal ripeness for consumption.

In the present study, environmental conditions and genetic background had an important role on fruit firmness according to the results of the ANOVA analysis of variance test. It was observed that the composition of the treatments can have an effect on this quality indicator, however without an obvious trend. Foliar treatments increased the value of this parameter. The results being significant in the case of fertilization variant 2 - Codamix (32.38 HPE units) compared to the unfertilized variant (30.50 HPE units) (Figure 3). Analyzing the influence of the variety on the firmness of the fruit texture, the best results were recorded for the Duke variety (37.27 HPE units) followed by the Pastel variety (34.17 HPE units). The lowest value of fructo-textural firmness was registered by the varieties Simultan (27.35 HPE units) and Elliott (27.60 HPE units).

Fruit color plays a key role in the marketing plan (Cömert et al., 2020), with consumers preferring vivid, brighter shades of fruit. Consumer perception of fruit color can be an indicator of taste. According to Vangdal et al. (2010) fruits become lighter (L* brightness increases), redder (a* color feature increases) and bluer (b* color feature decreases) as they approach technical maturity (Vangdal et al., 2010). In blueberries, the blue color is due to the anthocyanin pigments in the epicarp and the structure and quality of the wax on the skin (Saftner et al., 2008). The color brightness index L* between the fertilization variants there were significant differences on the numerical values of the brightness L* in the CIELa*b* color space, according to the Duncan test, presented in Table 2. In the unfertilized fruits (V1), the numerical

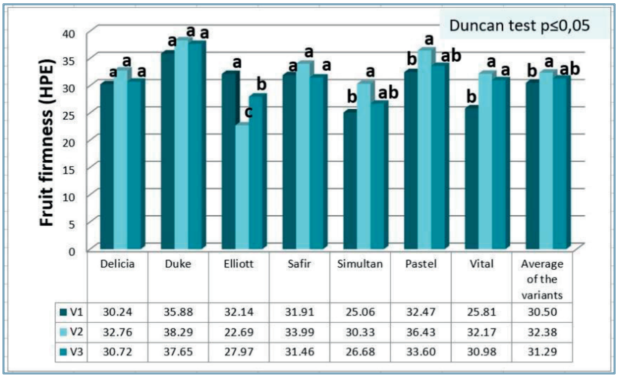


Figure 3. The effect of the fertilization option on the firmness of the berries according to the variety in the Argeș area 2020-2022

value of the brightness of the color being lower. It is found that the type of foliar fertilizers is significant on this characteristic. The mean of the variants indicates two series of homogeneous values. The brightness of the color of the fruit fertilized with organic products being higher. Among the varieties, Duke stands out with the higher average numerical value of brightness (32.64) significantly higher than the other varieties as can be seen from Table 2 (average of the varieties).

Table 2. The effect of the fertilization variant (V1-V3) on the lightness index L* of the color (from the CIELAB color space) of blueberries according to the variety

Variety/Variant	**Delicia**	**Duke**	**Elliott**	**Safir**
V1 - untreated	25.92 ± 1.49^{b}	31.55 ± 4.30^{b}	28.89 ± 2.56^{b}	26.05 ± 2.32^{b}
V2 - Codamix (0.25%)	27.53 ± 2.62^{ab}	34.76 ± 2.51^{a}	30.72 ± 1.35^{a}	29.28 ± 2.70^{a}
V3 - Ecoaminoalga (0.25%)	28.60 ± 1.77^{a}	31.61 ± 1.69^{b}	31.21 ± 1.86^{a}	27.01 ± 1.43^{b}
Average of the variety	27.40 ± 2.36^{c}	32.64 ± 3.30^{a}	30.27 ± 2.06^{b}	27.45 ± 2.53^{c}
Variety/Variant	**Simultan**	**Pastel**	**Vital**	**Average of the variants**
V1 - untreated	26.06 ± 2.38^{b}	27.70 ± 1.74^{a}	25.06 ± 1.23^{a}	27.32 ± 3.14^{b}
V2 - Codamix (0.25%)	28.84 ± 1.29^{a}	27.09 ± 3.37^{a}	25.11 ± 1.5^{3a}	29.00 ± 3.61^{a}
V3 - Ecoaminoalga (0.25%)	26.77 ± 2.79^{b}	26.43 ± 3.3^{2a}	26.35 ± 2.34^{a}	28.29 ± 3.01^{a}
Average of the variety	27.22 ± 2.47^{c}	27.07 ± 2.86^{c}	25.51 ± 1.81^{d}	28.21 ± 3.32

Color coordinate a*. The numerical values of the chromatic parameter a* in the color space CIELa*b*, were also significantly influenced by the fertilization system according to the Duncan test, presented in Table 3. The average values in the right column are grouped into two classes of homogeneous values (a and b). Organically fertilized fruits are redder. Among the varieties, Safir stands out with the higher numerical value of the red shade (3.70) as shown by the average of the variety.

Color coordinate b*. As can be seen with the other color indicators, the fruits obtained by organic fertilization had the chromatic parameter b* lower than the unfertilized ones (Table 4). Codamix organic fertilizer positively influencing this parameter significantly. It can be said that organic foliar fertilizers have a beneficial role on fruit color. Genetic background has a very significant role in determining fruit color.

The soluble dry matter content of the fruits is an important parameter that correlates with the texture and composition of the fruits (Kamiloglu, 2011).

Table 3. The effect of the fertilization variant (V1-V3) on the color coordinate a* (from the CIELAB color space) of the fruit epicarp according to the variety

Variety/Variant	Delicia	Duke	Elliott	Safir
V1 - untreated	0.56 ± 0.38[a]	0.47 ± 0.32[c]	0.19 ± 0.40[a]	0.85 ± 0.71[b]
V2 - Codamix (0.25%)	0.66 ± 0.31[a]	0.36 ± 0.03[d]	0.52 ± 0.44[a]	7.25 ± 5.31[a]
V3 - Ecoaminoalga (0.25%)	0.81 ± 0.32[a]	0.82 ± 0.54[a]	0.52 ± 0.01[a]	2.01 ± 1.41[b]
Average of the variety	0.69 ± 0.34[c]	0.55 ± 0.37[c]	0.41 ± 0.33[c]	3.70 ± 2.48[a]
Variety/Variant	**Simultan**	**Pastel**	**Vital**	**Average of the variants**
V1 - untreated	0.52 ± 0.16[a]	3.21 ± 1.69[a]	0.91 ± 0.82[b]	1.10 ± 0.64[a]
V2 - Codamix (0.25%)	0.80 ± 0.87[a]	1.37 ± 1.13[c]	1.75 ± 1.14[ab]	1.78 ± 1.32[a]
V3 - Ecoaminoalga (0.25%)	0.22 ± 0.20[a]	1.10 ± 0.71[c]	2.92 ± 2.16[a]	1.19 ± 0.76[a]
Average of the variety	0.51 ± 0.41[c]	1.89 ± 1.18[b]	1.86 ± 1.62[b]	1.36 ± 0.96

Table 4. The effect of the fertilization variant (V1-V3) and on the chromatic parameter b* (from the CIELAB color space) of blueberries according to the variety

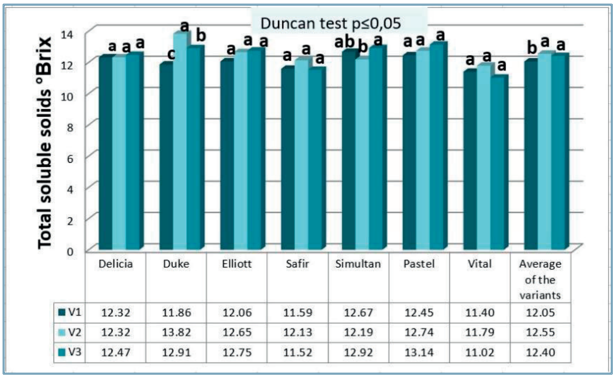
Variety/Variant	Delicia	Duke	Elliott	Safir
V1 - untreated	-1.49 ± 1.39[a]	-3.30 ± 0.85[a]	-3.68 ± 1.13[b]	-1.59 ± 0.73[a]
V2 - Codamix (0.25%)	-1.69 ± 0.98[a]	-9.17 ± 7.51[b]	-4.32 ± 1.02[a]	-3.19 ± 1.61[b]
V3 - Ecoaminoalga (0.25%)	-2.20 ± 1.77[b]	-3.65 ± 0.57[a]	-4,11 ± 0.91[a]	-1.99 ± 0.59[ab]
Average of the variety	-1.80 ± 1.39[a]	-5.37 ± 2.98[c]	-4,03 ± 1.01[c]	-2.26 ± 1.18[b]
Variety/Variant	**Simultan**	**Pastel**	**Vital**	**Average of the variants**
V1 - untreated	-0.80 ± 1.30[a]	-2.09 ± 0.54[a]	-1.32 ± 0.43[a]	-2.04 ± 1.23[a]
V2 - Codamix (0.25%)	-2.78 ± 1.20[b]	-2.20 ± 1.69[a]	-0.76 ± 0.97[a]	-3.40 ± 2.14[b]
V3 - Ecoaminoalga (0.25%)	-1.38 ± 1.25[ab]	-2.94 ± 1.51[a]	-0.76 ± 1.69[a]	-2.43 ± 1.54[a]
Average of the variety	-1.65 ± 1.42[a]	-2.41 ± 1.32[b]	-0.95 ± 1.10[a]	-2.63 ± 1.64

Testing the influence of experimental factors on the content of soluble dry matter in all seven varieties studied, we emphasize the following (Figure 4): on the average of the variants, the highest content of soluble dry matter was observed in variant 2 - Codamix (12.55°Brix) closely followed by variant 3 - Ecoaminoalga (12.40°Brix).

On the average of the varieties, the Duke and Pastel varieties recorded the highest content of soluble solids from the fruits (12.86°Brix and 12.77°Brix respectively), with significant differences compared to the Safir and Vital varieties (11.75°Brix respectively 11.41°Brix). Koort et al. (2020) obtained a soluble dry matter content that varied between 9.6 and 11.9°Brix in the blueberry cultivars studied with a significant effect due to the organic treatments administered.

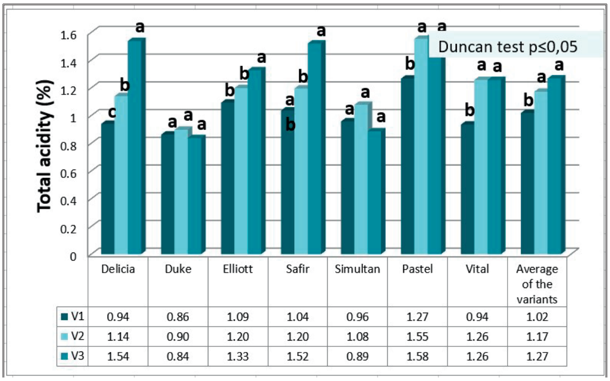
Ongoing research studies the influence of various cultivation technologies on the biochemical quality of blueberry fruits (Wang et al., 2008). Along with other phytochemical compounds, the soluble solids content contributes to fruit flavor.

Total titratable acidity. The influence of the three studied factors (genotypic differences, experimental fertilization models and environmental conditions) on the content of organic acids (expressed as malic acid) in the fruits was significant according to the ANOVA analysis of variance test. From this point of view, the effect of genetic factors was very significant, at a rate of 33.7%. The effect of crop practices was also highly significant at 16.0%. The 15.2% variation determined by environmental conditions in the three years of experimentation is highly significant (p=0.000). According to the Duncan multiple variation test, the tested fertilization models had a significant impact on the total acidity of the fruits, on the average of the variants (right column) two classes of homogeneous values (a and b) were observed (Figure 5).



	Delicia	Duke	Elliott	Safir	Simultan	Pastel	Vital	Average of the variants
V1	12.32	11.86	12.06	11.59	12.67	12.45	11.40	12.05
V2	12.32	13.82	12.65	12.13	12.19	12.74	11.79	12.55
V3	12.47	12.91	12.75	11.52	12.92	13.14	11.02	12.40

Figure 4. The effect of the fertilization option on the content of soluble solids in blueberries depending on the variety in the Argeș area 2020-2022



	Delicia	Duke	Elliott	Safir	Simultan	Pastel	Vital	Average of the variants
V1	0.94	0.86	1.09	1.04	0.96	1.27	0.94	1.02
V2	1.14	0.90	1.20	1.20	1.08	1.55	1.26	1.17
V3	1.54	0.84	1.33	1.52	0.89	1.58	1.26	1.27

Figure 5. The effect of the fertilization option on the content of organic acids in blueberries depending on the variety in the Argeș area 2020-2022.

Both foliar fertilizers applied caused a significant increase in fruit acidity (1.17% and 1.27%) compared to the non-fertilized version (1.02%); although in the fruits of the Duke and Simultan varieties, no statistically guaranteed variations were observed on this fruit quality parameter.
From the point of view of the variety, the highest values of malic acid in blueberry fruits were obtained in the Pastel and Safir varieties (1.47% and 1.29%, respectively) and the lowest in the Duke variety (0.83%) and Simultan (0.97%), the differences being very significant. The data obtained are consistent with those in the specialized literature. Lee and Kader, (2000) mention that fruit acidity can also vary according to post-harvest handling procedures.
Total sugar content. Along with the level of organic acids, the content of sugars in fruits has an essential role on the organoleptic quality of fruits (Li et al., 2020). The metabolism of this compound can be influenced by cultivation technologies, climatic conditions, the genetic baggage of the variety, soil properties, the position of the fruits in the bush, etc. (Davidescu, 1999; Gündoğdu, 2019).
On average, over the three years of the study, the total sugar content of blueberries was significantly influenced by cropping systems. The organic fertilization variants had a beneficial effect on the value of this quality indicator compared to the non-fertilized variant (Figure 6), on the average of the variants, the appearance of two classes of homogeneous values (a and b) can be observed.
Among the varieties, Pastel had the richest content of total sugars (11.18%) and the Safir variety the lowest (7.73%). The beneficial effect of the accumulation of sugars in the biomass of horticultural products was also observed in other studies (Kirina et al., 2020; Paraschiv & Hoza, 2022).

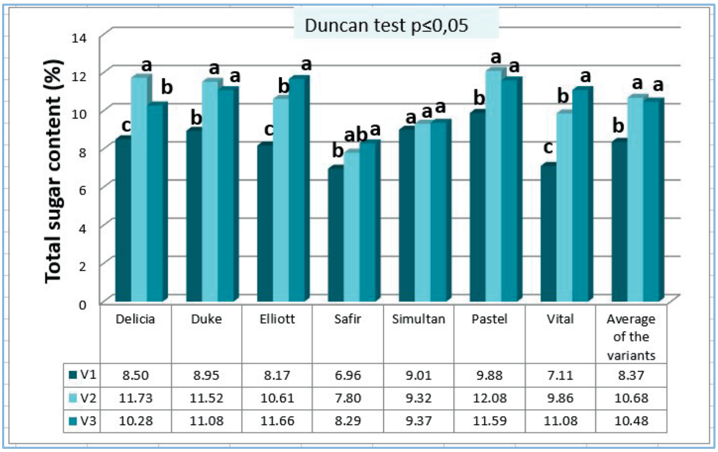


Figure 6. The effect of the fertilization option on the total sugar content of blueberries depending on the variety in the Argeș area 2020-2022

Tables 5 and 6 show the interdependence between the characteristics analyzed. Fruit production per plant correlated negatively, distinctly significantly with fruit firmness (Table 5), the correlation coefficient being r=- 0.208. There is a positive but insignificant correlation between fruit production per plant and average fruit mass, which means that production would be higher in plants with larger fruits. There is a significant correlation between fruit mass and color. Brighter fruits (L*) have greater mass (correlation coefficient r=0.397). Also, bluer fruits (the value of b* decreases) are larger (r=-0.178). The distinctly significant negative correlation between color brightness and the color coordinate b* indicates that bluer fruits are brighter, meaning that brightness increases as the fruit matures.

The content of soluble solid substance correlates significantly negatively with the value of the color coordinate b (Table 6) the correlation coefficient being r=-0.161 which means that the bluer fruits (the value of the coordinate b decreases) have more soluble solid substances.

Table 5. Pearson correlations coefficients for the productivity and the main biophysical parameters of the fruits (mass, firmness and color)

Pearson Correlation		Yield (g/bush)	Fruit weight (g)	Firmness (Hpe)	L*	a*	b*
Yield (g/bush)		1					
Fruit weight (g)		0.063	1				
Fruit firmness (HPE)		-0.208(**)	0.060	1			
L		-0.184(*)	0.397(**)	0.056	1		
a*		-0.009	0.053	0.023	0.017	1	
b*		0.075	-0.178(*)	0.012	-0.470(**)	0.072	1
	Sig.	0.309	0.010	0.869	0.000	0.297	
	N	213	213	213	213	213	213

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 6. Pearson correlations coefficients for the quality indicators for the studied blueberry varieties

Pearson Correlation		Total sugar content (%)	Total acidity (%)	Total soluble solids (°Brix)	L*	a*	b*
Total sugar content (%)		1					
Total acidity (%)		0.057	1				
Total soluble solids (°Brix)		0.249	0.206	1			
L*		0.231	-0.168	0.114	1		
a*		0.126	-0.195	-0.086	0.017	1	
b*		-0.049	-0.187	-0.161(*)	-0.470(**)	0.072	1
	Sig.	0.839	0.430	0.020	0.000	0.297	
	N	60	60	213	213	213	213

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

Different cultivation technologies significantly influence fruit production. Considering the positive effect of organic foliar fertilizers on productivity and fruit quality, this practice would be advisable.

Certain fruit quality parameters can be significantly improved by applied foliar fertilizers (mass, firmness, fruit color).

The variation in the content of organic substances in the fruits of the seven blueberry varieties ('Delicia', 'Duke', 'Elliott', 'Safir', 'Simultan', 'Pastel' and 'Vital') is dependent on the genetic background, on agronomic practices, the composition of applied foliar fertilizers and climatic conditions.

Of the two organic fertilizers used, Ecoaminoalga fertilizer is recommended for increasing crop yield, and both products are indicated for increasing quality.

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EFFECTS OF CYTOKININS IN *CORYLUS AVELLANA* L. MICROPROPAGATION

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Abstract

This study was carried out to investigate the effect of three cytokines [6-benzyladenine (BA), meta-Topoline (mT), and zeatin (Z)] on the in vitro propagation of C. avellana. The culture medium used in all in vitro culture stages was McCown's Woody Plant Medium (WPM) supplemented with 100 mg/l Sequestrene 138.3% (w/v) sugar, solidified with 5 g/l. Plant agar and pH 5.8. At the initiation stage, single-node mini cuttings were inoculated on WPM medium supplemented with 2 mg/l Z, and the initiation percentage was 66.66%. The highest proliferation rate (2.21 ± 0.11) was observed on the culture medium supplemented with 4 mg/l BA followed by the culture medium supplemented with 4 mg/l Z (1.93 ± 0.03). The shortest shoots were measured on WPM medium with 4 mg/l BA, with an average length of 1.81 ± 0.08 cm, and the longest shoots (3.46 ± 0.02 cm) were obtained on WPM medium with 4 mg/l Z. The in vitro rooting percentage was 81.66 ± 0.39% when using the WPM medium with 1 mg/l Z. The rooted plants obtained in vitro showed an acclimation rate of 97.66%, and out of these, 96% survived under greenhouse conditions.

Key words: *Barcelona hazelnut, 6-benziladedine, meta-Topoline, zeatin, proliferation rate*

INTRODUCTION

The hazelnut is considered a recalcitrant species under *in vitro* growth conditions because it exhibits a low rate of initiation and multiplication, insufficient shoots elongation, and high rates of microbial contamination (Yu & Reed, 1995; Nas, 2004; Nas & Read, 2004; Prando et al., 2014: Ellena et al., 2018; Sgueglia et al., 2019; Silvestri et al., 2020; Neda et al., 2020). Many basal media and their modifications have been used for hazelnut micropropagation: Anderson medium (Anderson, 1984) (Yu & Reed, 1993), DKW (Driver & Kunijuki, 1984) (Yu & Reed, 1993; Nas, 2004; Tegg et al., 2016; Ellena et al., 2018), Murashige and Skoog medium (MS) (Murashige & Skoog, 1962) (Nas, 2004; Silvestri et al., 2020), WPM (Lloyd and McCown, 1980) (Clapa et al., 2019; Kiran et al., 2021).
Furthermore, several studies have been conducted with the aim of developing tissue culture media to improve the micropropagation systems for different hazelnut genotypes (Bacchetta et al., 2008; Hand and Reed, 2014; Hand et al., 2014; Akin et al., 2017).
Among plant growth regulators (PGRs), cytokinins played a crucial role in shoot development (Wu et al., 2021). Thus, exogenous and endogenous cytokinins used in different concentrations in plant tissue culture were considered closely associated with shoot organogenesis (García-Ramírez, 2023). Consequently, some cytokinins were tested in order to obtain maximum values recorded on proliferation rates during the *in vitro* multiplication stage of hazelnut. In several studies, N6 benzyladenine (BA) was used at different concentrations for *in vitro* multiplication at hazelnut cultivars such as Montebello (Daminano et al., 2005), Dorris, Jefferson, and Sacajawea (Hand & Reed, 2014), Dorris, Wepster and Zeta (Akin et al 2017), Carrello, Ghirara, Minnulara, and Panottara (Sgueglia et al., 2019) and also local hazelnuts varieties collected from different locations in Turkey (Kiran et al., 2021). Other cytokinins used in hazelnut micropropagation

were: kinetin, iso-pentenyladenine (IPA) (Thomson and Deering, 2011), and zeatin (Bacchetta et al., 2005; Thomson & Deering, 2011).

Conversely, some studies have shown that the choice of iron source significantly influences the success of hazelnut *in vitro* culture. Fe-EDTA represents the most used iron source in hazelnut micropropagation (Silvestri et al., 2020). Sequestrene 138 has also been a source of iron successfully used in *in vitro* culture of hazelnut (Yu & Reed, 1993; Yu & Reed, 1995; Thomson & Deering, 2011; Hand & Reed, 2014; Hand et al., 2014; Clapa et al., 2019).

Although in recent years, there have been significant advancements in the development of protocols for micropropagation of *C. avellana*, proliferation rates have remained relatively low. Therefore, the aim of this study was to investigate the influence of three cytokines (BA, mT, and Z) used in the in vitro multiplication stage of C. avellana and to develop an efficient and reproducible micropropagation protocol adapted for the Barcelona hazelnut variety.

MATERIALS AND METHODS

Barcelona cv. was used for this study. All the experiments were carried out using McCown's Woody Plant Medium (WPM) supplemented with 100 mg/l Sequestrene 138.3% (w/v) sugar, and solidified with 5 g/l Plant agar. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH and/or 0.1 N HCl before adding the gelling agent and autoclaving at 121ºC for 20 min. The *in vitro* cultures were incubated in the growth chamber at 16 h photoperiod, 32.4 μmol $m^{-2}s^{-1}$ light intensity (Philips CorePro LEDtube 1200 mm 16W865 CG, 1600lm Cool Daylight) and temperature of 23 ± 3°C. The chemicals used were purchased from Duchefa Biochemie B.V., Haarlem, The Netherlands.

In vitro culture initiation

Young plants (Figure 1a), with juvenile growths, were used for the initiation of *in vitro* cultures. In April, shoot fragments measuring 5-7 cm in length with a 1-1.5 mm diameter were harvested. After removing the leaves, mini-cuttings were washed thoroughly, first with running tap water and the distilled water with three drops of Twen 20 for 10 minutes on a magnetic stirrer plate to eliminate all the dust and impurities. After that, the shoot fragments were disinfected with a bleach solution of 20% ACE (Procter and Gamble, București, Romania; <5% active ingredient) for 20 min followed by triple-rinse with sterile distilled water. The single-node explants of hazelnut were inoculated on WPM medium supplemented with 2 mg/l Z and gelled with 5 g/l (w/v) Plant agar in glass test tubes (11.5 × 2 cm ø) containing 5 ml sterile medium (Figure 1 b, c.). In the *in vitro* initiation stage, 60 explants were inoculated into the culture media and, after one month of culture, the shoot growing percentage and contamination rate was calculated.

In order to establish and provide plant stock for subsequent *in vitro* multiplication experiments, the regenerated shoots were further multiplied at two month intervals through two passages on WPM medium supplemented with 1 mg/l Z. In the stabilization stage, 720 mL (v/v) culture jars (13.5 × 9 cm ø) with screw caps were used as culture vessels. The screw caps were fitted with ventilation holes (4 mm ø) and with an autoclavable plastic sponge (18 mm × 18 mm). In each culture jar, 100 mL (v/v) of sterile medium was dispensed and ten explants (1-1.5 cm in length, containing 2-3 nodes) were inoculated.

In vitro shoots multiplication

In the multiplication stage, the effect of three cytokinins was examined: 6-benzyladenine (BA), meta-Topoline (mT), and zeatin (Z). Each was added to the culture medium at a concentration of 4 mg/l before autoclaving. In each jar (similar to those described above) containing 100 ml of medium, 10 mini cuttings with 2-3 nodes from the culture medium supplemented with 1 mg/l Z were inoculated. After two months of culture, the shoot length and proliferation rate were calculated.

In vitro rooting and acclimatization

Rooting was tested *in vitro* on WPM supplemented with 1 mg/l Z and after three months, the percentage of rooting, the average shoot length, the average no. of roots, and roots length were measured and calculated from 108 plantlets (3 jars × 12 plantlets/jar in 3 replicates). Subsequently, *in vitro* rooted plants were acclimatized in a floating hydroponic system according to the method described by Clapa et al (2013) for one week. Then, the plantlets were planted in a mix of peat and perlite (3:1, v/v) (Klasmann, TS3 Medium Basic Standard, with pH = 6) in mini-greenhouses (Versay, T1, sizes 39 × 25 × 7.5 cm, PVC). The percentage of acclimated plants was then recorded after 20 days of culture in mini-greenhouses.

The rooted and acclimatized hazelnuts plantlets were then transplanted into pots (VQB 9 x 9 x 9.5 black, SC BLONDY ROMANIA SRL 540390 Tg-Mures, Romania) containing peat-based potting mix (Klassman TS3) and kept in greenhouse conditions (21 ± 4°C), under natural photoperiod conditions. The survival rates (%) under greenhouse conditions were calculated after 30 days.

Data Analysis

The *in vitro* experiments were carried out in a completely randomized design (CRD) and one-way ANOVA was performed to check the differences between the experimental variants. When the null hypothesis was rejected, Tukey's HSD test ($p < 0.05$) was used to determine the differences between the means. The values presented are means ± S.E.

RESULTS AND DISCUSSIONS

Previous research has shown that the *in vitro* initiate potential of hazelnuts cultivars is generally difficult due to microbial contaminants and decontamination techniques (Bacchetta et al., 2008; Sgueglia et al., 2019; Silvestri et al., 2020). In a study conducted by Andrés et al. (2002) endogenous levels of indole-3-acetic acid, abscisic acid, and cytokinins (Z-type: dihydrozeatin, dihydrozeatin riboside, zeatin, and zeatin riboside; iP-type: N6 -isopentenyl adenine and N6-isopentenyl adenosine), were determined in leaves of hazelnut. The aforementioned study shows that the ratios of iP-type/Z-type cytokinins were low in the analysed samples from autumn and spring leaves, while they were high in the juvenile and forced outgrowth samples. Thus, in our study, young plants, with juvenile growths, were used for the initiation of *in vitro* cultures and the cytokinin used was zeatin. Our results show that during the initiation stage, when single-node micro-cuttings were inoculated on WPM medium supplemented with 100 mg/l Sequestrene 138 and 2 mg/l Z, 66.66% (Figure 1 b, c) of the explants were viable, 21.66% did not show any development or growth, and 11.66% were infected with contaminants.

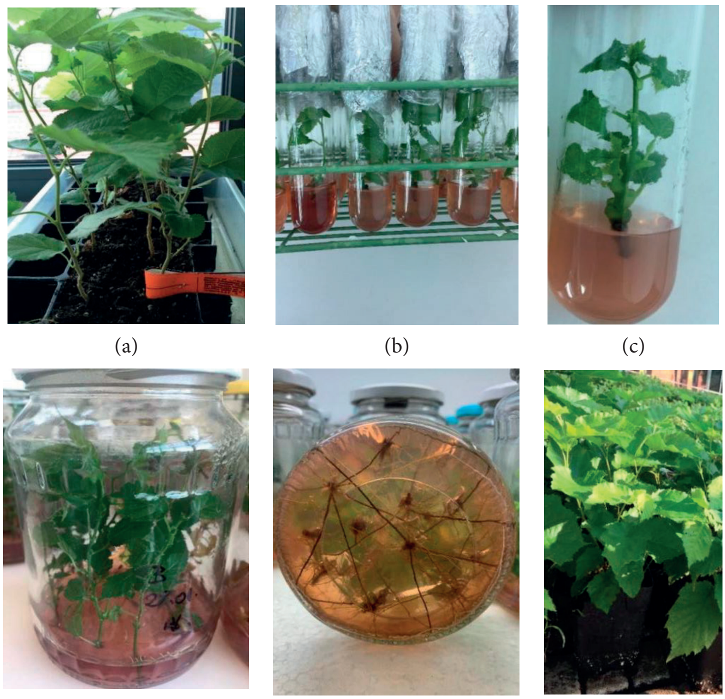
Previous studies have demonstrated an improvement in the regeneration potential of hazelnut cultivars within micropropagation systems. In a study conducted by Silvestri et al. (2020), an efficient *in vitro* establishment stage was performed for the Tonda Gentile Romana hazelnut variety. The researchers utilized a half-strength MS medium supplemented with 20 g/L sucrose, 6 mg/l 6-benzyl aminopurine, 0.1 mg/l naphthalene acetic acid, 0.1 mg/l thidiazuron, and 0.55% plant agar. Different concentrations of $CuSO_4{\cdot}5H_2O$ (0.0, 1.25, 2.5, and 5 mg/L) were added to the medium. The addition of copper sulfate was found to significantly reduce bacterial contamination in the culture medium. Specifically, 2.5 mg/l $CuSO_4{\cdot}5H_2O$ resulted in a significant decrease in contamination frequency during the establishment phase while maintaining a high rate of bud sprouting. On the other hand, the highest concentration (5.0 mg/l) of copper sulfate significantly reduced contamination rates but had a negative impact on bud sprouting. Therefore, this concentration was deemed unsuitable for the purpose of the study.

Sgueglia et al. (2019) conducted a study in which they utilized axillary buds taken from 1-year-old twigs of mature plants. The buds were subjected to decontamination treatments involving sodium hypochlorite and sodium merthiolate for either 35 or 40 minutes. The researchers observed that the 40+40 minute treatment positively reduced explant contamination in all cultivars. However, it also led to a higher incidence of necrosis overall, particularly in the Carrello cultivar (50%) and the Panottara cultivar (46.7%). In terms of bud survival, the 40+40 minute treatment yielded the highest rates in Minnulara (26.7%) and Ghirara (20%), while the 35 + 35 minute treatment was more effective for Carrello (33.3%) and Panottara (23.3%).

The shoots obtained during the initiation phase of hazelnut *in vitro* culture were subsequently transferred to the same culture medium used for the initiation stage but with the addition of 1 mg/l Z. On this medium, the shoots exhibited robust growth and successfully developed roots. It is worth mentioning that the inoculums containing apical buds demonstrated higher growth compared to those with axillary buds. Additionally, the results of our study showed that the shoots obtained from apical buds rooted at a 100% rate. This indicates that the mini shoots with apical buds are more suitable for rooting compared to other types of explants in hazelnut micropropagation. (as depicted in Figure 1, panels d and e).

In contrast, the use of mT at a concentration of 4 mg/l did not lead to any proliferation, with only one shoot being generated from each inoculum. This suggests that mT may not be as effective as BA and Z in shoot multiplication in hazelnut cultures. (Figure 2 c, d, and Figure 3).

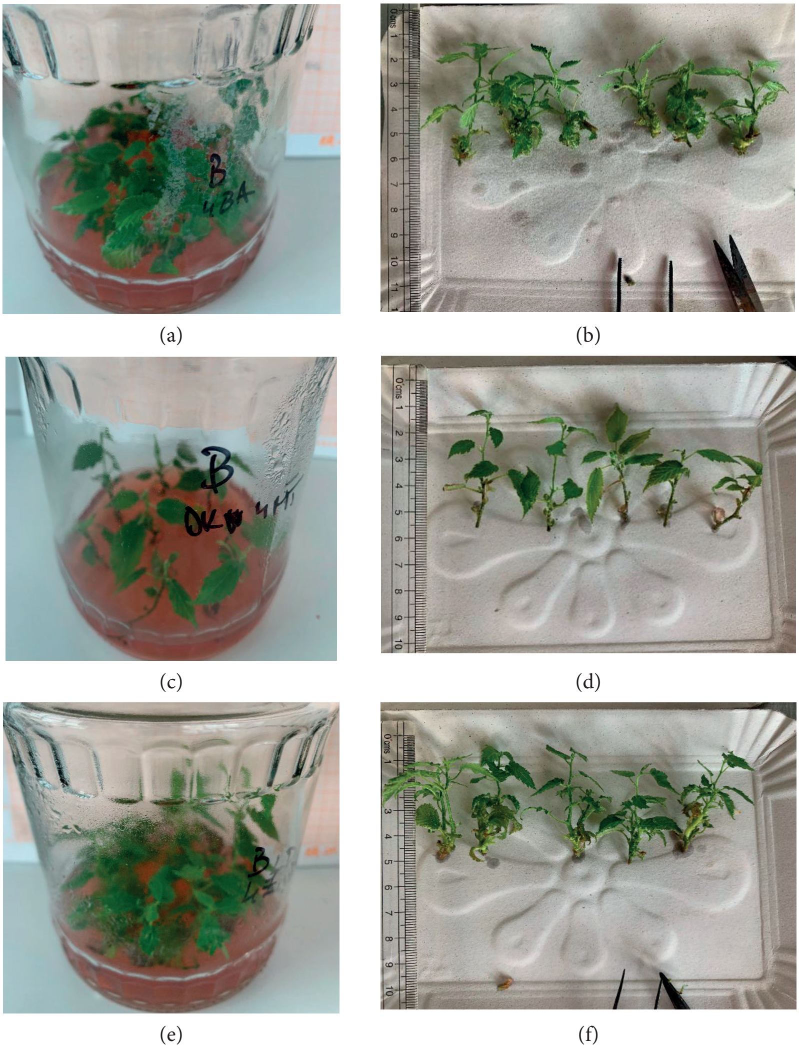
The highest proliferation rate, recorded as 2.21 ± 0.11, was observed on the culture medium supplemented with 4 mg/l BA (Figure 2 a, b) followed by the culture medium supplemented with 4 mg/l Z (Figure 2 e, f) which generated an average rate of proliferation of 1.93 ± 0.03. However, no significant differences were observed between the mean values of the multiplication rate for these two variants, as depicted in Figure 3. A significant portion of the literature emphasizes the positive outcomes of using BAP for *in vitro* shoot multiplication in hazel plants. For example, the multiplication rate of hazelnut cvs. Nonpareil and Tonda Gentile Romana were 3.2 respectively 3.1, on DKW medium supplemented with 3 mg/l BA (Yu & Reed, 1993). The effect of BA concentration on shoot formation was tested on the cultivar Montebello. Opposite of our results, the highest multiplication rates were obtained using concentrations of 1.5 and 2.0 mg /l BA. The shoots treated with 3.0 mg l^{-1} BA were highly hyperhydric (Damiano et al., 2005).



(a) (b) (c)

(d) (e) (f)

Figure 1. *In vitro* propagation of *C. avellana*, cv. Barcelona: (a) Plants used to initiate *in vitro* culture; (b, c) *In vitro* culture initiation on WPM+ 100 mg/l Sequestrene 138 +1 mg/l Z; (d) *In vitro* shoot proliferation on medium WPM+ 100 mg/l Sequestrene 138 +1 mg/l Z after 12 weeks of incubation; (e) *In vitro* rooting of shoots cultured on WPM+ 100 mg/l Sequestrene 138 +1 mg/l Z; (f) Plants acclimatized in the greenhouse



(a) (b) (c) (d) (e) (f)

Figure 2. *In vitro* shoot proliferation of *C. avellana*, cv. Barcelona on WPM medium supplemented with 100 mg/l Sequestrene 138 and different cytokinins and gelled with 5 g/l Plant agar after three months of incubation: (a, b) Shoot proliferation on media containing 4 mg/l BA; (c, d) Shoot proliferation on media containing 4 mg/l mT; (e, f) Shoot proliferation on media containing 4 mg/l Z.

Table 1. *In vitro* rooting and acclimatization data recorded in micropropagated plants of *C. avellana* cv. Barcelona

In vitro shoots and roots morphometry			*In vitro* rooting (%)	*Ex vitro* acclimatization (%)	Survival under greenhouse conditions (%)
Length of shoots (cm)	No. of roots	Length of roots (cm)			
6.35 ± 0.25	3.65 ± 0.36	2.10 ± 0.04	81.66 ± 0.39	97.66	96

Values shown are means ± SE.

Regarding the average length of the proliferated shoots, the shortest shoots were observed when using WPM medium supplemented with 4 mg/l BA, measuring an average length of 1.81 ± 0.08 cm. On the other hand, the longest shoots (3.46 ± 0.02 cm) were obtained when utilizing a WPM medium supplemented with 4 mg/l Z (Figure 3). Another study (Bacchetta et al., 2005) showed that zeatin (1 mg/L) was more suitable than BAP (0.5 mg/L) for shoot elongation.

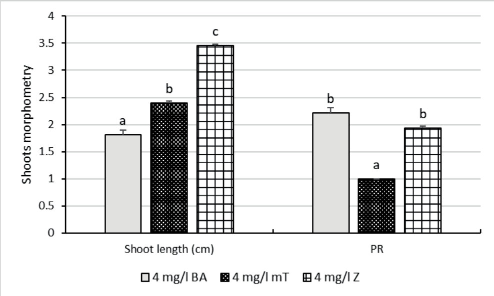


Figure 3. The effect of 6-benzylidene (BA), metatopolin (mT), and zeatin (Z) treatments on shoot proliferation in *C. avellana* cv. Barcelona after 12 weeks of *in vitro* culturing. Different lowercase letters above the bars indicate significant differences between the means of the same parameter according to Tukey's HSD test ($p<0.05$)

Therefore, the WPM culture medium supplemented with 100 mg/l Sequestrene 138 and 4 mg/l Z is the most suitable for shoot proliferation in the Barcelona hazelnut variety as it promotes the growth of longer shoots compared to 4 mg/l BA.

For the *in vitro* rooting of hazelnut shoots, different culture media were supplemented with indole-3-butyric acid (IBA), indoleacetic acid (IAA), or naphthaleneacetic acid (NAA) at various concentrations and combinations. In their study, Damiano et al. (2005) demonstrated that the best rooting response in the Montebello hazelnut variety was achieved with 9.8 μM (2 mg/l) of IBA, resulting in a rooting percentage of 79%. At similar concentrations of IAA and NAA, the rooting percentages were lower (71% and 65%, respectively).

In our study, on the WPM medium with 1 mg/l Z, 81.66 ± 0.39 % of the shoots were *in vitro* rooted while the average number of roots per plantlet was 3.65 ± 0.36 and the average root length was 2.10 ± 0.04 cm (Table 1). The plantlets rooted *in vitro* had a 97.66% acclimatization rate (in floating hydroculture followed by acclimatization in a mix of peat and perlite in mini-greenhouses) and 96% of the plants survived in greenhouse conditions (Table 1; Figure 1 d, e, f).

Our results show that among the tested cytokines, zeatin can be used in all stages of *in vitro* multiplication in the Barcelona hazelnut variety. This can be explained by the fact that juvenile hazelnut tissues as well as those with forced growth show a high morphogenetic potential, suggesting that the ratio of iP-type/Z-type cytokinins may be a good index of the *in vitro* potential of the hazelnut (Andrés et al., 2002).

CONCLUSIONS

Our research has highlighted that the WPM culture medium supplemented with 100 mg/l Sequestrene 138 and 1-4 mg/l Z can be used in all in vitro culture stages for hazelnut (*Corylus avellana* L.) cv. Barcelona. In the initiation stage, a 66.66% initiation rate was achieved on the WPM culture medium supplemented with 2 mg/l Z. The longest shoots, measuring 3.46 ± 0.02 cm, were obtained on the WPM culture medium supplemented with 4 mg/l Z, and an in vitro rooting percentage of 81.66 ± 0.39% was observed when using the WPM medium with 1 mg/l Z.

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THE EFFECT OF SOME FOLIAR FERTILIZERS ON THE BIOMETRIC CHARACTERISTICS OF THE FRUITS OF PEACH VARIETIES (*P. VULGARIS* L.) GROWN IN THE PEDOCLIMATIC CONDITIONS OF LUGOJ, TIMIȘ COUNTY

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Abstract

Foliar fertilizers play a very important role in obtaining quality harvests and present more and more benefits that increase the resistance of plants to diseases and pests and implicitly to reduce the number of treatments with fungicides and insecticides that have a negative impact on the environment. In order to carry out the research, two lesser-known peach varieties, 'Piros Magdalena' and 'Gold Dust' were studied. The varieties were treated with four foliar fertilizers in three different growth phenophases: the phenophase of intense shoot growth, the phenophase of fruit growth and before fruit ripening. The foliar products used were the following: Albit (organic product), Cropmax (organic product), Foliq N Universal and Solfert. Regarding the fruit mass, among the biological fertilizers, in both varieties, the best results were obtained with the Cropmax fertilizer, and among the chemical fertilizers, the best results were obtained with the Solfert fertilizer in the 'Gold Dust' variety, and with Foliq N Universal fertilizer in the 'Piros Magdalena' variety.

Key words: *fertilizer, peach, fruit mass, 'Gold Dust', 'Piros Magdalena'.*

INTRODUCTION

Fertilizer application is one of the essential cultural practices that play a very important role in obtaining quality harvests (Andreev et al., 2018; Jia et al., 1999). A combination of soil-applied and foliar applied fertilizers is more efficient and can lead to yield benefit and net income increase (Dixon, 2003; Gonzalez et al., 2008).

Moreover foliar fertilizers present more and more benefits that increase the resistance of plants to diseases and pests and implicitly to reduce the number of treatments with fungicides and insecticides that have a negative impact on the environment (Farahy et al., 2021; Iordănescu et al., 2023; Kuepper, 2003; Reuveni & Reuveni, 1998).

Persica vulgaris L. is a species that reacts well to fertilization, being a significant consumer of N and K (Damianov et al., 2022). In terms of production, the peach is the second-most significant temperate fruit crop globally after the apple and one of the most appreciated fruit (Manganaris et al., 2022; Olimpia et al., 2009).

Peach consumption has positive effects on one's health because they are rich in antioxidants, polyphenols, and carotenoids, which are all essential medicinal compounds. Peach consumption has also been linked to a number of therapeutic benefits, including impacts on the heart, chemoprevention, maintaining eye health, obesity, neurodegenerative diseases and antidiabetic activity (Bento et al., 2022; Byrne et al., 2007; Hussain et al., 2021; Noratto et al., 2014).

Considering that in a pre-purchase situation, the external appearance of the fruits has the strongest effect that determines a consumer's choice (Ali et al., 2021; Czarnocińska et al., 2003; Tarancón et al., 2021; Zhang et al., 2014), our research aims to study the effects of some foliar fertilizers on the biometric characteristics of the fruits of two peach varieties in order to enhance fruit quality.

MATERIALS AND METHODS

In order to carry out the research, two lesser-known peach varieties, 'Gold Dust' and 'Piros

Magdalena' (Figure 1 and Figure 2) were studied, which were treated with four foliar fertilizers in three different growth phenophases: the phenophase of intense shoot growth, the phenophase of fruit growth and before fruit ripening. Each variety was divided into four different groups, each group being treated in all phenophases with the same foliar fertilizer.

Figure 1. 'Gold Dust' variety

Figure 2. 'Piros Magdalena' variety

The foliar products used were the following: Albit (organic product) (100 ml/ha), Cropmax (organic product) (1.5 L/ha), Foliq N Universal (5 L/ha) and Solfert (4 kg/ha).

The experiment was conducted in the Lugoj fruit-tree nursery (45°42'22.1"N 21°51'36.1"E), during the year 2022.

The trees were planted in 2015 and are all grafted on Oradea peach rootstock and trained in a "vase-shape". The plantating distances consists in spacing of 4 m between rows and 4 m in the row.

The biometric characterization of the peach fruits involved the determination of their size (fruit height, large diameter, small diameter) and mass (with stone and without stone).

In order to analyze the specific parameters, were taken 15 fruit samples from each group of the two varieties. The fruits had been harvested at their optimal ripening stage.

Fruit height, large diameter and small diameter were determined with the digital caliper (Insize-1108, Loganville, GA, USA) and the mass of the fruits was measured using the analytical balance (Kern PES620-3M, Balingen, Germany).

Statistical calculations were performed using SAS Studio software SAS® Studio 3.8, applying One Way Anova and nonparametric Kruskal-Wallis test at a significance level of 0.05.

RESULTS AND DISCUSSIONS

The results determined for the examined parameters (fruit height, large diameter, small diameter, fruit mass and stone mass) are presented in Table 1, Table 2 and Figures 3-7.

Table 1. The influence of the fertilisers on the characteristics in the 'Gold Dust' variety

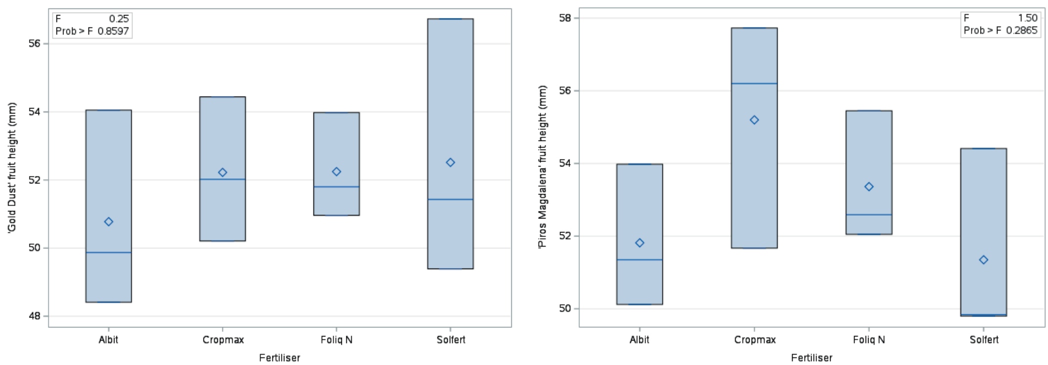
Fertiliser	Variable	Mean	Standard Deviation	Minimum	Maximum	Median
Albit	Fruit height (mm)	50.78	2.93	48.41	54.05	49.87
	Large diameter (mm)	55.20	2.56	53.44	58.13	54.02
	Small diameter (mm)	53.86	3.00	51.22	57.12	53.23
	Fruit mass (g)	92.09	18.10	77.91	112.48	85.89
	Stone mass (g)	12.51	1.57	10.87	13.99	12.68
Cropmax	Fruit height (mm)	52.22	2.12	50.21	54.44	52.02
	Large diameter (mm)	57.35	2.40	55.08	59.86	57.11
	Small diameter (mm)	55.55	2.10	54.13	57.97	54.56
	Fruit mass (g)	100.08	10.43	91.81	111.80	96.62
	Stone mass (g)	12.86	1.44	11.26	14.05	13.27
Foliq N	Fruit height (mm)	52.25	1.56	50.96	53.98	51.80
	Large diameter (mm)	58.05	3.16	54.68	60.95	58.52
	Small diameter (mm)	56.76	2.81	54.27	59.81	56.20
	Fruit mass (g)	101.84	10.22	90.86	111.09	103.56
	Stone mass (g)	12.86	0.54	12.25	13.26	13.08
Solfert	Fruit height (mm)	52.52	3.79	49.39	56.73	51.43
	Large diameter (mm)	58.45	7.40	52.78	66.82	55.75
	Small diameter (mm)	56.73	4.88	52.77	62.18	55.25
	Fruit mass (g)	104.57	32.02	81.80	141.18	90.72
	Stone mass (g)	11.81	1.93	9.93	13.78	11.73

Table 2. The influence of the fertilisers on the characteristics in the ‘Piros Magdalena’ variety

Fertiliser	Variable	Mean	Standard Deviation	Minimum	Maximum	Median
Albit	Fruit height (mm)	51.82	1.97	50.12	53.98	51.35
	Large diameter (mm)	57.39	3.01	55.41	60.85	55.90
	Small diameter (mm)	56.55	4.18	51.79	59.62	58.23
	Fruit mass (g)	96.34	19.36	80.52	117.93	90.57
	Stone mass (g)	12.08	0.22	11.94	12.33	11.96
Cropmax	Fruit height (mm)	55.20	3.15	51.67	57.73	56.20
	Large diameter (mm)	58.46	2.05	56.10	59.81	59.48
	Small diameter (mm)	56.40	2.97	53.14	58.95	57.12
	Fruit mass (g)	101.02	9.53	90.58	109.26	103.22
	Stone mass (g)	10.20	1.22	8.89	11.30	10.42
Foliq N	Fruit height (mm)	53.36	1.83	52.05	55.45	52.59
	Large diameter (mm)	57.36	3.19	53.69	59.42	58.98
	Small diameter (mm)	56.33	3.55	52.38	59.23	57.39
	Fruit mass (g)	100.43	15.26	83.20	112.22	105.88
	Stone mass (g)	9.97	1.19	9.01	11.30	9.61
Solfert	Fruit height (mm)	51.35	2.65	49.80	54.41	49.84
	Large diameter (mm)	55.66	4.09	51.10	58.99	56.89
	Small diameter (mm)	55.64	2.51	52.77	57.42	56.72
	Fruit mass (g)	93.69	17.23	75.34	109.53	96.19
	Stone mass (g)	9.85	1.13	8.65	10.89	10.01

Fruit height values (Figure 3)
‘Gold Dust‘ variety had values of the fruit height between 48.41 mm (Albit fertiliser) and 56.73 mm (Solfert fertiliser), with an experiment average of 51.94 mm, with no significant differences recorded. The best results (52.52 mm mean) were obtained with the Solfert fertiliser. Between the organic fertilisers the best result were obtained with the Cropmax fertiliser (52.22 mm mean).

Fruit height values in the ‘Piros Magdalena’ variety were between 49.80 mm (Solfert fertiliser) and 57.73 mm (Cropmax fertiliser), with an experiment average of 52.93 mm, with no significant differences recorded. As can be seen in Figure 3 **(b)** the best results regarding the fruit height were obtained with the Cropmax fertiliser (55.20 mm mean).

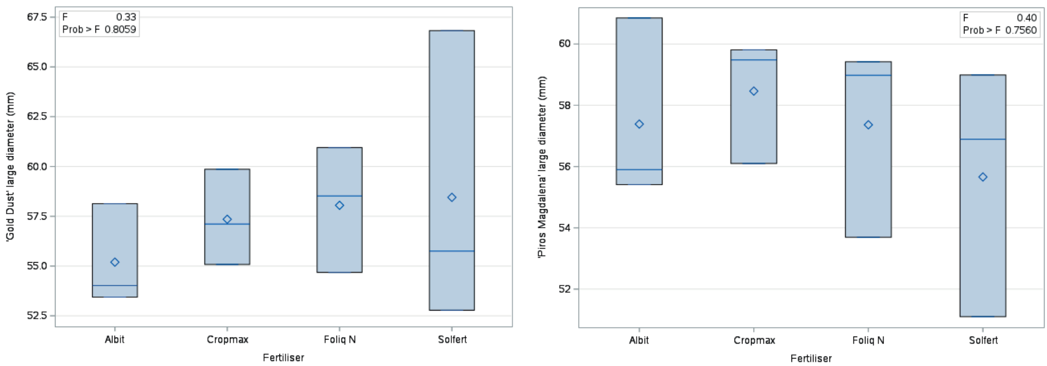


(a) **(b)**

Figure 3. The influence of the fertilisers on the fruit height: **(a)** ‘Gold Dust‘ variety; **(b)** ‘Piros Magdalena‘ variety

Fruit large diameter values (Figure 4)
‘Gold Dust‘ variety fruits had values of the large diameter between 52.78 mm (Solfert fertiliser) and 66.82 mm (Solfert fertiliser), with an experiment average of 57.26 mm, with no significant differences recorded. The best results (58.45 mm mean) were obtained with the Solfert fertiliser. Between the organic fertilisers, the best results were obtained with the Cropmax fertiliser (57.35 mm mean).

Values of the large diameter of the fruits in the ‘Piros Magadalena’ variety ranged between 51.10 mm (Solfert fertiliser) and 60.85 mm (Albit fertiliser) with an experiment average of 57.21 mm, the differences not being statistically significant.
The best results for this variety were obtained with the Albit fertiliser (57.39 mm mean).



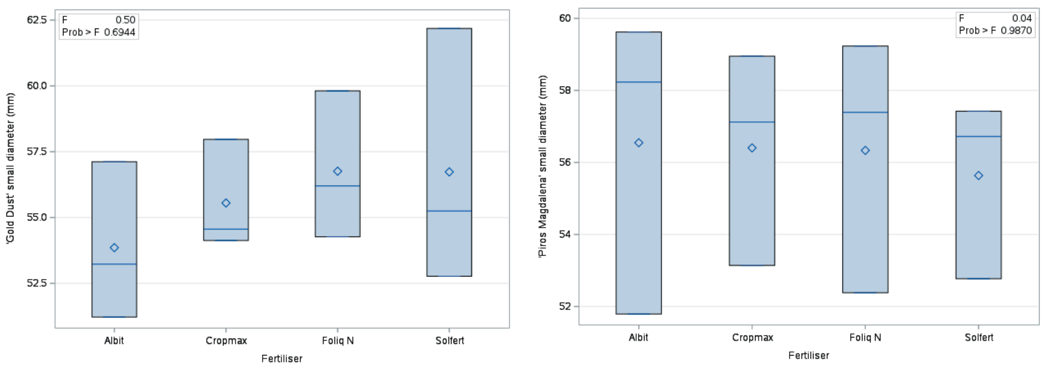
(a) **(b)**

Figure 4. The influence of the fertilisers on the large diameter: **(a)** 'Gold Dust' variety; **(b)** 'Piros Magdalena' variety

Fruit small diameter values (Figure 5)

'Gold Dust' variety fruits had values of the small diameter between 51.22 mm (Albit fertiliser) and 62.18 mm (Solfert fertiliser), with an experiment average of 55.72 mm, with no significant differences recorded. The best results (56.7 mm mean) were obtained with the Foliq N fertiliser. Between the organic fertilisers, the best results were obtained with the Cropmax fertiliser (55.55 mm mean).

Values of the small diameter of the fruits in the 'Piros Magadalena' variety ranged between 51.79 mm and 59.62 mm (Albit fertiliser), with an experiment average of 56.23 mm, the differences not being statistically significant. The best results for this variety were obtained with the Albit fertiliser (57.39 mm mean).



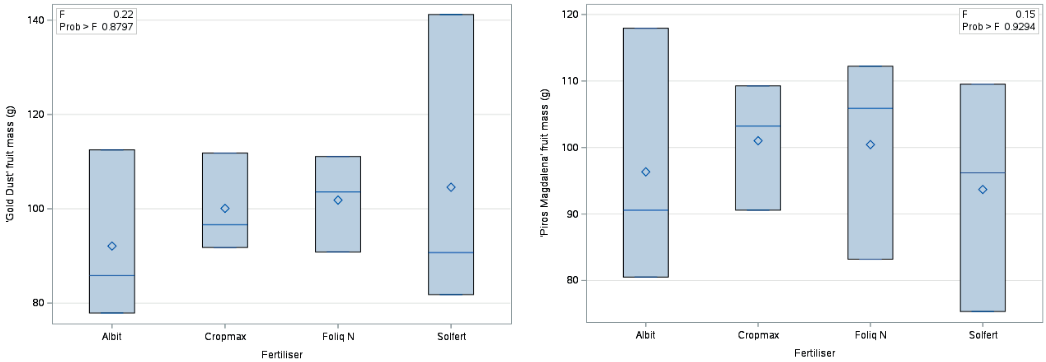
(a) **(b)**

Figure 5. The influence of the fertilisers on the small diameter: **(a)** 'Gold Dust' variety; **(b)** 'Piros Magdalena' variety

Fruit mass values (Figure 6)

'Gold Dust' variety had values of the fruit mass from 77.91 g (Albit fertiliser) to 141.18 g (Solfert fertiliser), with an experiment average of 99.64 g, with no significant differences. The best results (104.57 g mean) were achieved with the Solfert fertiliser. Among the organic fertilisers the best result were obtained with the Cropmax fertiliser (100.08 g mean).

Fruit mass values in the 'Piros Magadalena' were between 75.34 g (Solfert fertiliser) and 117.93 g (Albit fertiliser), with an experiment average of 97.87 g, with no significant differences recorded. The best results regardind the fruit mass were registered with the Cropmax fertiliser (101.02 g mean).



(a) **(b)**

Figure 6. The influence of the fertilisers on the fruit mass: **(a)** 'Gold Dust' variety; **(b)** 'Piros Magdalena' variety

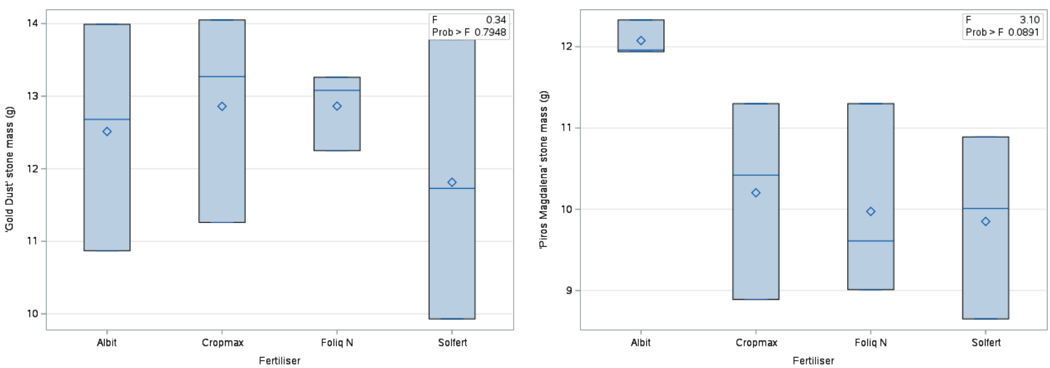
Stone mass values (Figure 7)

The lowest values of the stone mass were recorded in both varieties with the Solfert fertiliser.

'Gold Dust' variety had values of the stone mass from 9.93 g (Solfert fertiliser) to 14.05 g (Cropmax fertiliser), with an experiment average of 12.51 g, with no significant differences. Among the organic fertilisers the lowest values of the stone mass were obtained with the Albit fertiliser (12.51 g mean).

The values of the stone mass in the 'Piros Magdalena' variety were the closest ($p>0.0891$) to a p-value that can indicate a statistical difference between the foliar fertilisers. In this case, the studied parameter values were between 8.65 g (Solfert fertiliser) and 12.33 g (Albit fertiliser), with an experiment average of 10.52 g. As shown in Figure 7 **(b)**, the highest values of the stone mass were registered with the Albit fertiliser (12.08 g mean), with a very small standard deviation (± 0.22 g) and the lowest values with Solfert fertiliser (9.85 g mean).

Furthermore, to confirm the results obtained after applying the One Way Anova test, the non-parametric Kruskal-Wallis test was also applied for all the examined parameters. In all performed determinations, the p-value was higher than 0.05, which also indicates that the differences between the effect of foliar fertilisers are small in value and do not have statistical significance.



(a) **(b)**

Figure 7. The influence of the fertilisers on the stone mass: **(a)** 'Gold Dust' variety; **(b)** 'Piros Magdalena' variety

CONCLUSIONS

The effect of foliar fertilizers on biometric characteristics is different depending on the variety, however, in this case without statistical significance.

Regarding the fruit mass, the best results were obtained with the Solfert fertiliser in the 'Gold Dust' variety and with the Cropmax fertiliser in the 'Piros Magdalena' variety. Among the biological fertilizers, in both varieties, the best results were obtained with the Cropmax fertilizer, and among the chemical fertilisers, the best results were obtained with the Solfert fertilizer in the 'Gold Dust' variety, and with Foliq N Universal fertiliser in the 'Piros Magdalena' variety.

Future research is necessary, especially regarding the influence of the fertilisers on the internal characteristics of the fruits.

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CORRELATIONAL INTERCONNECTIONS BETWEEN VEGETATIVE AND REPRODUCTIVE PERFORMANCES IN RASPBERRY CULTIVAR 'TULAMEEN'

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Abstract

The scientific experiment was conducted in the period 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Bulgaria. The plantation was created in the autumn of 2016. The planting scheme of the plants is 3.00/0.50 m. The objective of the study is 'Tulameen' cultivar. Vegetative parameters were monitored: average number of shoots, average thickness and average height of shoots and reproductive: average fruit weight and yield per linear meter. The number of shoots had the highest values in the first year of the experiment (29.33), and their height (1.51 m) and thickness (7.60 mm) in the second year. The highest yield was recorded in the third year (1.48 kg/1 m^2). A high to very high positive correlation between shoot height and thickness was reported in all three years. In the first year, a high correlation dependence was registered between shoot height and yield (0.76) and negative in the second (-0.85) and third (-0.51) years of the experiment.

***Key words**: cultivar, fruit weight, raspberry, vegetative indicators, yield.*

INTRODUCTION

Raspberry production is traditional for the mountain and foot-hill regions of Bulgaria, which is directly related to a more efficient use of arable land. To be economically profitable, a correct choice of cultivars, quality planting material, suitable soil and climate conditions and intensive cultivation technologies are necessary (Georgiev et al., 2013; Serbezova, 2019; Georgiev, 2021). On the other hand, the raspberry as a fruit crop is also attractive to farmers because of its quick return on the invested financial means to create plantations, as it bears fruit already in the second year after planting, and the full fruit bearing begins in the third year.

Currently, a sustainable trend is noticed towards the use of high-yielding and adaptable cultivars, application of modern technologies for productive fruit cultivation, ensuring optimal, regular and high yields is noticed (Leposavić et al., 2013; Sønsteby et al., 2013; Domozetova, 2014). The productivity of raspberries is determined by the number of shoots per 1 linear meter and their development, as well as by the number and weight of their fruits per shrub. The study and improvement of their economic qualities and traits marks continuous progress in science.

The aim of the scientific work is to study the correlational interconnections between the vegetative and reproductive indicators in the 'Tulameen' raspberry cultivar grown in the Troyan region.

MATERIALS AND METHODS

The scientific experiment was conducted in the 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The object of the study was the introduced 'Tulameen' raspberry cultivar, which was a result of crossing *Nootka* x *Glen Prosen* (http://omafra.gov.on.ca/english/crops/facts/raspvarc.htm#Jewel).

The planting scheme is 3.00/0.50 m. The plants are grown under irrigated conditions with drip irrigation. Row spacings are naturally grassed, as the necessary mowing was conducted, while the intra-row area is maintained in black fallow by tillage.

'Tulameen' is a promising large-fruited raspberry cultivar with high-quality fruits that ripen later and over a longer period. The cultivar is distinguished by relatively firm fruits, which is why it has good transportability and is used both for processing into various products and for fresh consumption (Daubeny and Anderson, 1991; Ivanova et al., 2012; Serbezova, 2019). The planting material used was obtained by *in vitro* technology, providing healthy plant specimens with an even growth habit.

The following indicators were studied:

- average number of shoots per linear meter;
- average height of shoots (m);
- average thickness of shoots (mm), measured 10 cm from the soil surface;
- average fruit weight (g);
- average yield of 1 m^2 (kg).

The experiment was set in variant with six replications, each was one linear meter of the intra-row area.

The plants are planted in pits measuring 0.30/0.30/0.30 m, with the application of granulated chicken manure of 0.200 kg. The methodology for studying plant resources in fruit plants was used to report the indicators (Nedev et al., 1979). The data were processed by correlation analysis, the software product MS Excel – 2010 was used.

RESULTS AND DISCUSSIONS

The results presented in Table 1 show that the highest average number of shoots was in the first experimental year (29.33), and in the following two years the values were in close range. Leposavić et al. (2013) in a similar experiment with 5 raspberry cultivars obtained 5.43 average number of shoots/m from 'Tulameen' in a 0.33 x 2.5 m planting scheme under the conditions of Western Serbia.

The average number of shoots for the period is 24.39. The highest values for the average height of the shoots were recorded in the second year with 1.51 m. The smallest plant height was recorded in the first and third year with very little difference between the values. Analogous results were obtained for the average thickness of the shoots. In the second experimental year, the thickness reached 7.60 mm, it was significantly smaller in the first year (6.09 mm) and in the third year (6.51 mm). Regarding the average fruit weight, the highest value was reported in the first year (2.83 g) and with close results in the following two years. The average fruit weight for the period was 2.42 g. In our previous studies (Georgiev et al., 2013), 193.98 cm height, 8.78 mm thickness of raspberry shoots and 3.54 g fruit weight of the tested cultivar were recorded. Leposavić et al. (2013) reported fruit weight (largeness) of 4.28 g and a yield per shoot 375 g, as the impact of agroecological conditions being decisive, especially in years with extreme temperatures in the studied cultivar. The highest yield was recorded in the third year (1.48 kg/1 m^2), and the lowest was recorded in the first year (0.80 kg/1m^2). For the three-year period, the average yield was 1.10 kg/1 m^2.

Table 1. Vegetative and reproductive indicators of 'Tulameen' cultivar for the period 2018-2020

Average number of shoots per 1m^2	Average shoot height (m)	Average shoot thickness (mm)	Average fruit weight (g)	Yield (kg/1 m^2)
2018				
29.33	1.34	6.09	2.83	0.80
2019				
22.33	1.51	7.60	2.24	1.03
2020				
21.50	1.39	6.51	2.19	1.48
Average value for the period 2018-2020				
24.39	1.41	6.73	2.42	1.10

In the first year, a high correlation between shoot height and thickness (0.80) and height and yield (0.76) was observed. A significant correlation was recorded between thickness and yield (0.67) (Table 2).

Table 2. Correlation interconnections between vegetative and reproductive performances in raspberry cultivar 'Tulameen' in 2018

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	-0.19	1		
Thickness	-0.47	0.80	1	
Yield	0.26	0.76	0.67	1

In the following year, a very high correlation dependence between the height and the thickness of the shoots was reported (0.93). A high but negative dependence between height and yield (-0.85) and a very high negative dependence between thickness and yield (-0.95) was registered (Table 3).

Table 3. Correlational interconnections between vegetative and reproductive performances in raspberry cultivar 'Tulameen' in 2019

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	-0.37	1		
Thickness	-0.02	0.93	1	
Yield	-0.08	-0.85	-0.95	1

In the third year, again a very high correlation was observed for height with shoot thickness (0.94) and significant between shoot number and yield (0.58) (Table 4). A significant negative dependence was reported between height and yield (-0.51) and a moderate negative dependence between thickness and yield (-0.42).

Table 4. Correlational interconnections between vegetative and reproductive performances in raspberry cultivar 'Tulameen' in 2020

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	0.03	1		
Thickness	0.17	0.94	1	
Yield	0.58	-0.51	-0.42	1

On average for the period, a very high correlation was reported between shoot height and thickness (1.00) and a high but negative correlation between shoot number and yield (-0.82). Significant but negative correlation dependences were registered between the number of shoots with the height (-0.66) and with the thickness (-0.64) (Table 5).

Table 5. Correlational interconnections between vegetative and reproductive performances in raspberry cultivar 'Tulameen' average for 2018-2020

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	-0.66	1		
Thickness	-0.64	1.00	1	
Yield	-0.82	0.11	0.09	1

CONCLUSIONS

Based on the obtained results for the vegetative and reproductive characteristics of the introduced raspberry cultivar 'Tulameen', combined with the soil and climate conditions of the region, the applied agricultural techniques and the cultivar specificity, it can be assumed that the Troyan region is suitable for its cultivation.

'Tulameen' has very good shoot-forming ability. The shoots are thin, which requires the plants to be grown on a supporting structure. A high to very high correlation between shoot height and thickness was reported. In the second and third years, a significant to high negative correlation was recorded between shoot height and yield and a moderate to very high negative correlation between thickness and yield. The cultivar is characterized by average-sized fruits.

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EVALUATION OF SOME SWEET CHERRY CULTIVARS GRAFTED ON 'GISELA 6' ROOTSTOCK

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Abstract

The results regarding the evaluation of the trunk cross-sectional area of the scion, the rootstock and the grafting point and the calculation of the morphological index of thickening of the grafting zone in 5 sweet cherry cultivars ('Early Lory', 'Early Bigi', 'Grace Star', 'Regina' and 'Kordia'), grafted on 'Gisela 6' rootstock, are presented in this paper. The research was carried out in a commercial orchard located in Orodel commune, Dolj County, South-West part of Romania. The orchard was established in 2013, with a planting distance of 4 x 1.8 m. Based on the results obtained, the morphological index of thickening of the grafting area fluctuated significantly depending on the cultivar, reaching the highest value in 'Regina' cv. (0.012), the lowest being recorded in 'Kordia' and 'Early Lory' cv. (0.008). The largest differences between the rootstock and the grafting point were recorded in 'Grace Star' cv. (approx. 147 cm^2), and the largest differences between the grafting point and the scion were recorded in 'Early Lory' and 'Kordia' cv. (approx. 32 cm^2).

Key words: *compatibility, symbionts, trunk cross-sectional area.*

INTRODUCTION

Sweet cherry (*Prunus avium* L.), belongs to the genus *Prunus*, family *Rosaceae* (Aglar & Yildiz, 2014) and is cultivated mainly in countries with a temperate climate. Its fleshy drupes are among the most valued fruits and are mainly consumed fresh, but also processed in various forms (jam, marmalade, juice, compote, candied fruit) (Wani et al., 2014). Rootstocks have been used for more than 2000 years as a method of vegetative propagation of fruit tree species (Sitarek, 2008). Sweet cherry production has greatly advanced as a result of new selections of low-vigor rootstocks, the use of modern methods of protection of orchards against environmental factors, harvesting, handling and storage of fruits (Musacchi et al., 2015). Jakab-Ilyefalvi and Chiorean (2022) mention the worldwide use in sweet cherry culture of optimal management systems and cultivar × rootstock combinations to control the morphological characters of the plant and implicitly the production yield. The most commonly used rootstocks for cherry are 'Mazzard', 'Mahaleb', 'Weiroot 13', 'Colt', 'Gisela 5', 'Gisela 6', 'Gisela 12' (Long & Kaiser, 2010), 'Krymsk 5' and 'Krymsk 6'. Rootstock influences growth, production, but also fruit size and quality (Martins et al., 2021; Aglar et al., 2016; Aglar & Yildiz, 2014; Whiting et al., 2005). The use of semi-dwarf rootstocks is preferred in orchards where a higher production per unit area is desired (Aglar & Yildiz, 2014). Choosing an appropriate density according to the type of rootstock used and maintenance works are important cultural management strategies that improve the quality of an orchard (Neilsen et al., 2010). Trunk cross-sectional area (TCSA) is also influenced by water regime (used irrigation systems), soil treatments and tillage (Neilsen et al., 2010). The 'Gisela' dwarf rootstock series, developed at Liebig University in Giessen, Germany, induces early production and the current trend towards higher densities in stone and pome orchards calls for the adoption of more efficient management systems (Musacchi et al., 2015). 'Gisela 6' rootstock, exhibits a medium high vigor, induces precocity, it is suitable for the vast majority of soil types, but requires adequate drainage (Long & Kaiser, 2010). Hrotkó (2008) mentions the insufficient existing data on cultivar × rootstock interactions in sweet cherry, although dwarf rootstocks are among the most widely used. López-Ortega et al. (2016) confirm, following the study carried out in Jumilla (Murcia, Spain), the influence of rootstocks ('Adara', 'Gisela 5', 'Gisela 6', 'Mariana 2624', 'Ma × Ma 14', 'Mayor', 'Pikú 1', 'Pikú 3', 'Pikú 4', 'Saint Lucie GF 64') on the yield of 'Newstar' cultivar and implicitly on the size, acidity, color and firmness of the sweet cherry fruits. Sansavini and Lugli (2014) mention 'Gisela 6' rootstock as being adapted to hot climate conditions, but it requires suitable cultivars, planting distances, appropriate management systems, and to be successful, in addition to an appropriate cultivar × rootstock combination, the yield of the crop depends on the vigor, photosynthetic efficiency and nutritional balance of the plants. The same authors exemplify 'Regina' cultivar grafted on 'Gisela 6', as having larger, sweeter and firmer fruits compared to the same cultivar grafted on 'W158'. The present work proposed the analysis of the degree of compatibility of five sweet cherry cultivars grafted on 'Gisela 6' rootstock in a commercial orchard in the South-West part of Romania.

MATERIALS AND METHODS

Materials

The experience was located in Orodel commune, Cornu village (44°13'N 23°16'E), Dolj County, South-West part of Romania. The study was carried out in 2022, on the cultivars 'Early Lory', 'Early Bigi', 'Grace Star', 'Regina' and 'Kordia' (trees in the 9th year since planting) grafted on the semi-dwarf rootstock 'Gisela 6'. Planting distances are 4 m between rows and 1.8 m between plants per row, with a density of 1389 trees/ha. The shape of the canopy is slender spindle with a support system and anti-hail protection. The irrigation method used in the plantation is drip irrigation. Regarding the pedological data according to the studies carried out, the plantation is located on typical preluvosol.

Research area

From a climatic point of view, the area is characterized by an average annual temperature of 10.9°C, with a minimum monthly average of -0.9°C in January, and the highest in July with 23.1°C. The rainfall regime is characterized by average annual precipitation of approx. 530 mm, the largest amount corresponding to the month of May with approx. 73 mm and the smallest amount corresponding to the month of February with approx. 2.5 mm. Within the plantation, the prevailing winds are from the North-West and North-East.

Research method

To highlight the differences in vigor between the two partners (scion × rootstock), the method described by Zamfirescu (2022) was used. The trunk diameter was measured 5 cm above and below the grafting point, as well as at the level of the grafting point. The difference in thickening between the two symbionts was determined based on the calculation of the trunk cross-section area of scion (SA), rootstock (SP) and grafting point (SÎ), by applying the formula:

- $TCSA = (D/2)^2 \times 3.14$, in which: TCSA = trunk cross sectional area (cm^2); D = trunk diameter (cm).

After performing the three measurements, the morphological thickening index of the grafting zone MTI (morphological thickening index) was calculated as the result of the ratio between the trunk cross-sectional area of the scion, rootstock and the grafting point: MTI = SA/SP/SÎ.

The obtained data were statistically processed in the IBM SPSS Statistics 26 program and represent the mean, standard deviation, limits of variation and coefficient of variability. One-way ANOVA followed by Duncan's Multiple Range Test at $p < 0.05$ were used.

RESULTS AND DISCUSSIONS

The statistical data of the trunk cross-sectional area at the grafting point, below and above are presented in Table 1. From the analysis of the data, it appears that the cultivars 'Early Lory' and 'Early Bigi' recorded the highest values in terms of the measurement below the grafting point (SP), respectively 108.55 cm^2 and 107.85 cm^2. The lowest value (75.77 cm^2) was identified in 'Regina' cultivar for the same characteristic, i.e., 1.42 times lower than in 'Early Lory' cultivar. Regarding the data obtained when determining the grafting point (SÎ), 'Grace Star' cultivar recorded the highest value (235.37 cm^2) and the lowest was also identified at 'Regina' cultivar (147.14 cm^2). For this feature as well, a 1.59 times difference between extreme mean values emerges. Regarding the results obtained at the measurement above the grafting point (SA), there were significant differences, 'Grace Star' cv. (215.14 cm^2) registering the highest value and 'Regina' cv. (132.64 cm^2) the lowest value, i.e., of 1.62 times less. Usenik et al. (2017) reported the greatest differences in trunk cross-sectional area above and below the grafting point in sweet cherry grafted on 'Gisela 6' (compared to 'Gisela 5', 'P-HLC', 'Piku 1' and 'Weiroot 158') in the study conducted in Slovenia. The greatest difference between the rootstock and the grafting point (Figure 1) was 147.07 cm^2 corresponding to 'Grace Star' cv. and the lowest value corresponding to 'Regina' cv. with 71.37 cm^2. Regarding the differences between the grafting point and scion (Figure 1), 'Early Lory' and 'Kordia' cv. with close values (31.76 cm^2 and 31.63 cm^2) and 'Regina' cv. with the lowest value of 14.23 cm^2. The coefficient of variation had values between 16.51 and 24.69%, which represents an average and high variability between the analyzed individuals.

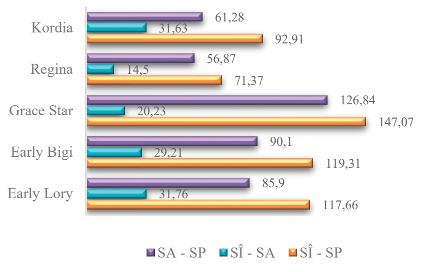

Figure 1. The differences between the trunk cross-sectional area of the grafting point (SÎ), rootstock (SP) and scion (SA) (cm^2)

The difference in thickening between rootstocks and scion had an average value of 84.19 cm^2, the biggest differences being in the case of the combination with 'Grace Star' cv. (126.84 cm^2), and the smallest differences at 'Regina' cv. (56.87 cm^2) (Figure 1). In all scion × rootstock combinations it was found that the trunk cross-sectional area above the grafting point (SA) was less than on the grafting point (SÎ) and greater than below (SP). The further the value of trunk cross-sectional area in the symbiont grafting point is compared to the values of the trunk cross-sectional area 5 cm above and below the contact zone of the symbionts, the lower the degree of compatibility of the combination (Asănică & Tudor, 2011; Mladin et al., 2006). According to Narandžić and Ljubojević (2023), trunk cross-sectional area measurements are frequently used to assess tree vigor and growth characteristics. Biško et al. (2017) mention 'Gisela 6' rootstock as incompatible with 'Regina' and 'Kordia' cultivars (compared to the same cultivars grafted on 'Gisela 5', 'Piku 1' and 'PHL-C' following the study carried out in the North-Western Croatia on very acidic soil, using the drip irrigation system.

The assessment of the grafting compatibility of the 5 sweet cherry cultivars in relation to the rootstock was carried out by calculating the morphological thickening index of the grafting area (Table 2), resulting from the ratio between trunk cross-sectional area of the rootstock (SP), scion (SA) and the grafting point (SÎ), and it was concluded that the morphological thickening index of the grafting zone in all 5 sweet cherry cultivars associated with 'Gisela 6' rootstock had low values, and the differences between the trunk cross-sectional area of the scion and the trunk section trunk cross-sectional area of the rootstock were very large (Figures 1 and 2), which indicates a reduced level of compatibility between the two symbionts.

According to the values of the morphological thickening index (Zamfirescu, 2022), grafting compatibility can be of three types: very good compatibility (type A) when the values of this index are greater than 0.330 and tend to 1.000; good compatibility (type B) when the morphological index values are between 0.300 and 0.330; poor compatibility (type C) when the values of the morphological index are lower than 0.300. As can be seen in Table 2, the highest morphological thickening index was calculated for 'Regina' cultivar (0.012), about 13% higher compared to 'Grace Star', but 31-33% higher compared to the group of cultivars 'Kordia', 'Early Bigi' and 'Early Lory'. Although there is poor compatibility (Figure 2) between partners (rootstocks/scion), canopy shape, maintenance work, climatic factors, applied irrigation system, soil quality also influence the vigor and productivity of the studied sweet cherry cultivars.

Table 1. Statistical descriptors (mean, standard deviation, extreme values and variation coefficient) of TCSA - trunk cross-sectional area (cm^2)

Measuring point	Cultivar	Mean ± SD*	Min.-Max.	CV (%)
Below grafting point (SP)	'Early Lory'	108.55 ± 25.10^a	49.71-150.50	23.12
	'Early Bigi'	107.85 ± 22.34^a	60.15-143.66	20.71
	'Grace Star'	88.30 ± 16.90^b	63.25-112.45	19.14
	'Regina'	75.77 ± 13.32^b	59.28-100.24	17.58
	'Kordia'	102.21 ± 16.33^a	84.01-150.50	15.98
At grafting point (SÎ)	'Early Lory'	226.21 ± 39.82^a	147.06-295.96	17.60
	'Early Bigi'	227.16 ± 44.60^a	133.70-310.69	19.63
	'Grace Star'	235.37 ± 36.64^a	183.25-295.96	15.57
	'Regina'	147.14 ± 36.33^c	100.24-215.07	24.69
	'Kordia'	195.12 ± 35.09^b	153.98-300.83	17.98
Above grafting point (SA)	'Early Lory'	194.45 ± 43.11^a	111.85-286.33	22.17
	'Early Bigi'	197.95 ± 38.46^a	114.85-262.97	19.43
	'Grace Star'	215.14 ± 37.31^a	161.06-273.13	17.34
	'Regina'	132.64 ± 31.57^c	91.94-181.73	23.80
	'Kordia'	163.49 ± 26.99^b	127.26-223.42	16.51

*SD- Standard Deviation; CV%-coefficient of variation. For each measurement point, different letters placed next to the values indicate significant differences between them

Table 2. Descriptive statistics of the morphological thickening index (MTI) of the graft area

Descriptive statistics	'Early Lory'	'Early Bigi'	'Grace Star'	'Regina'	'Kordia'
Mean ± SD*	0.008 ± 0.002^c	0.009 ± 0.002^c	0.011 ± 0.002^b	0.012 ± 0.002^a	0.008 ± 0.002^c
Min.-Max.	0.005-0.015	0.006-0.014	0.008-0.014	0.009-0.017	0.005-0.011
CV (%)	25.00	22.22	18.18	16.67	25.00

*SD- Standard Deviation; CV%-coefficient of variation. For each measurement point, different letters placed next to the values indicate significant differences between them

'Regina' 'Early Lory' 'Grace Star' 'Early Bigi' 'Kordia'

Figure 2 Grafting point of the five sweet cherry cultivars grafted on 'Gisela 6' rootstock

CONCLUSIONS

The combinations of 'Early Lory', 'Early Bigi', 'Grace Star', 'Regina' and 'Kordia', grafted on 'Gisela 6' rootstock, showed a highly disharmonious relationship between the rootstock, the scion and the grafting point. According to the values of the morphological index of thickening of the grafting zone, the compatibility is weak, in the order 'Early Lory' - 'Kordia' - 'Early Bigi' - 'Grace Star' - 'Regina'.

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ASSESSMENT OF FRUIT QUALITY AND BIOCHEMICAL COMPOUNDS OF SOME BLUEBERRY HYBRIDS

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Abstract

Vaccinium corymbosum fruits are used as raw material for the food industry. It is known that the highbush berries, compared to lowbush blueberries, are richer in dry matter and total sugar content. During 2021-2022, at the Researsch Institute for Fruit Growing Pitești-Mărăcineni, Romania was organized a field trial with two genotypes and their progenies. Data were collected for: average fruit weight,size index, firmness, ph,total soluble solid, total sugar content, vitamin C, total polyphenol, flavonoid, antocyanin, lycopene and β-carotene content. The aim of this study was to compare the analysis of the fruit quality in hybrids of 'Simultan' and 'Northblue' cutivars. The results showed significant differences between progenies. The fruit weight varied between 0.82 and 2.05 g, the firmness oscillated between 8.13 and 28.27 N, total soluble solids reached a maximum of 17.43° BRIX, size index varied between 8.13 to 28.27, vitamin C content varied from 3.48 to 13,49 mg/100 g. Total polyphenol, lycopene and β-carotene content averaged 1296.66 mg GAE/100 G, 0.10 mg and 0.42 mg. For the blueberry breeding programme, biochemical evaluation of hybrids characteristics is an important objective.

***Key words**: hybrids, biochemical compounds, highbush blueberry.*

INTRODUCTION

In the last years, the recent global interest in the consumption of berries with high levels of functional properties and nutraceuticals compounds is gaining momentum (Zimmer et al., 2014). The highbush blueberry (*Vaccinium corymbosum)* is original to North America and their content in nutrients exceeds that of lowbush blueberry or rabbiteye blueberry. (Korkak, 1988; Finn et al., 1993).

The blueberries are one of the most important functional and nutraceutical foods in our diets (Kahkonen et al., 2001) and it is were consumed worldwide. It is known that blueberries (*Vaccinium corymbosum)* are an execellent source of bioactive compounds, such as polyphenols, flavonoids, tannins, anthocyanins

In Romania, highbush blueberry was introduced for the first time in 1968 at the Research Institute for Fruit Growing Pitesti-Maracineni (Botez et al., 1984). For optimal growth and productivity high bush blueberry needs well drained acid soils (pH $_{H2O}$ 4.8-5.5) with the pH adjustment and additional organic matter before planting is necessary .The blueberry breeding program started in 1983 and were obtained varieties for adaptation in different ecological condition, prolonged shelf-life of blueberries, quantified by the berries firmness, technological flow and the content of bioactive compounds.

The highbush breeding activity at the Research Institute fot Fruit Growing Pitesti-Maracineni, achieved 9 cultivars: 'Azur', 'Safir', 'Augusta', 'Delicia', 'Simultan', 'Lax', 'Pastel', 'Prod' and 'Vital'.

'Simultan' and 'Northblue' cv. were selected for breeding program due to earliness and the special qualities of the fruits.

In the light of this, the purpose of our study was the analysis of the fruit quality (berry weight, firmness, size index, total soluble solids) and biochemical characteristics (total sugar contents, vitamin C, total polyphenol, flavonoid, antocyanin, lycopene and β-carotene content) of blueberry progenies obtained from 'Simultan' × 'Northblue' cv. for new cultivars with more aroma, size and taste.

MATERIALS AND METHODS

Chemicals and Reagents

Folin-Ciocalteu's phenol reagent, 2,4,6-Tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium nitrite, aluminium chloride, methanol, acetone, n-hexane, ethanol, citrate/acetate buffer, glucose, galli acid, catechin, vitamin C, metaphosphoric acid, acetic acid, hydrochloric acid, concentrated sulphuric acid were purchased the reagents used in the study were obtained from Merck, Darmstadt, Germany

Plant material

The biological material was represented by 20 promising blueberry progenies, tested in an experimental seedling plot with their parental forms as control. A number of 22 genotypes were studied (2 cultivars and 20 progenies).

The fruits of two cultivars of highbush, namely 'Simultan' obtained from open pollination of 'Spartan' cv. and 'Northblue' cv. was selected in 1973 from a cross of G × Ashworth (*Vaccinium corymbosum)* × US3 - paternal selection, (*Vaccinium angustifolium).*

The experiment was set up at the Research Institute Growing Pitesti, within the Genetic and Breeding Department in 2019. The genotypes were planted at a distance of 3 m × 1 m on a mixture of soil and peat. The samples were harvested and immediately analyzed.

Determination of average weight and chemical analysis

Following quality parameters and biochemical analyzes were determined: berry weight, size index, firmness, pH, total soluble solid,total polyphenols, total flavonoids, antocyanin, vitamin C content, total sugar content and lycopene and β-carotene levels.

The average fruit weight was determined by weighing of 50 fruits for each variety using HL-400 digital. The index size of the fruit was calculated by formula: (height + large diameter + small diameter)/3, (Botu et al., 1997). The fruit firmness was determined for each sample with a penetrometer Bareiss HPE II Fff nondestructive test, with a measuring surface of 0.25 cm². pH were determined in fruit flesh juice using a digital pH-metter.

The soluble solids (TSS) content was determined as °Brix (°Bx) with digital refractometer (Kern, ORF 45BM, Germany).

Determination of total polyphenols content (TPC) was determined according to the methodology suggested by Matić et al., 2017.

The results were expressed as mg gallic acid equivalent (GAE)/100 g dry weight of vegetal material.

The total flavonoids content (TFC) was determined according to the methodology suggested by Tudor-Radu et al., 2016. The results were expressed as mg catechin equivalent (CE)/100 g dry weight of vegetal material.

The level of total anthocyanins pigments (TAC) was determined according to the methodology suggested by Di Stefano and Cravero, 1989. The results were expressed as cyaniding-3glucoside mg/100 g dry weight of vegetal material (C 3 - G mg 100 g).

The total sugar content was determined using the colorimetric method and the methodology suggested by Dubois et al., 1956.

The results were expressed as g glucose/100 g dry weight of vegetal material.

Vitamin C content was estimated according to the colorimetric method and the methodology suggested by Omaye et al., the vitamin C content was determined by using 2,6-dichloroindophenol (DCPIP), at pH 3-4.5.The results were expressed in mg vitamin C/100 g dry weight of vegetal material.

The lycopene and β-carotene content were determined according to the methodology proposed by Tudor-Radu et al., by the carotenoids extraction in a mixture of hexane: ethanol: acetone. The results were expressed in mg lycopene/100 g dry weight of vegetal material and mg β-carotene/100 g dry weight of vegetal material.

Statistical analysis was performed using IBM SPSS 14 program (SPSS Inc., Chicago, IL, USA). All results were statistically evaluated by analysis of variance (ANOVA). Differences between cultivars were highlighted through Duncan's multiple test range ($p< 0.005$). Graphical representations were performed with Microsoft Office Word 2007.

RESULTS AND DISCUSSIONS

On the ensemble of the 22 genotypes from the experiment, tables 1-3 reflect close biochemical traits of the berries

As presented in Table 1, the average berry weight oscillated between 0.82 g for 16-2-1 hybrid and 2.05 g for 16-2-26 hybrid. The control ranged between 1.93 g 'Simultan' cv. and 2.04 g 'Northblue' cv.

In the case of the size index, the hybrid 16-2-1 had the highest average compared to the other genotypes studied (Table 1).

Analyzing the average values of the all three years of the study, in the case of firmness, the the highest value was recorded by 'Simultan' cv. 30.22 N, followed by the hybrid 16-2-13 with the value 28.27 N.

The pH (table 1) recorded normal values for the blueberry berries, oscillated between 3.08 for the hybrid 16-2-6 and 3.99 for the hybrid 16-2-17.

Total soluble solids (TSS) content reached a maximum of 17.63°Bx for the control 'Simultan' cv., and the hybrid 16-2-1 reached of 17.63°Bx. Similar data of the soluble solids content of the harvested fruits was in Guasca, Colombia was in a range of 12.4 to 14.5 °Brix (Cortes et al., 2016).

The results reported that the total phenolic content of genitors varied from 505.66 mg GAE/100 g for 'Northblue' cv. to 668.44 mg GAE/100 g 'Simultan' cv., while their progenies ranged from 225 to 1,296.67 mg GAE/100 g. Similar results, 455.01 mg GAE/100 g for 'Duke' cv. and 474.184 mg GAE/100 g for 'Hannah's choice'cv. reported by Ciucu et al., 2021.

Total flavonoid content in blueberry fruits of 'Simultan' and 'Northblue'cultivars and their progenies hybrids varied between 87.00 and 221.00 mg/100 g, having a mean value of 188.87 mg/100 g. It could be observed that most of the 'Simultan x Northblue' hybrids had flavonoid contents higher than their genitors (195.04 mg/100 g, for 'Simultan' and 211.39 mg CE/100 g for 'Northblue') and 16-2-17 hybrid was remarked (221.00 mg CE/100 g), followed by 16-2-24 (203.67 mg CE/100 g).

The total sugar content is an important biochemical marker used to determine the quality of sweetness berries (Okan et al., 2019) and sugars determine the organoleptic traits (Li et al., 2020). TSC (Table. 2) of the fruits varies between 4.76 g glucose/100 g for 16-2-3 hybrid and 12.67 g glucose/100 g for 16-2-14 hybrid. While the genitors registered the values 7.40 for 'Simultan' cv. and 8.09 for 'Northblue' cv.

Table 1. The average values of berry weight, size index, firmness, °Brix and pH at 22 blueberry genotypes

Genotypes	Average berry weight (g)	Size index	Firmness (N)	TSS (°Brix)	pH
16-2-1	$0.82\pm0.24^{g*}$	0.85 ± 0.12^{abc}	21.73 ± 4.02^{abcd}	17.43 ± 0.68^{a}	3.58 ± 0.07^{abcd}
16-2-2	1.38 ± 0.35^{de}	0.75 ± 0.02^{bcd}	17.3 ± 1.31^{bcd}	14.2 ± 0.26^{cde}	3.67 ± 0.1^{abcd}
16-2-3	1.65 ± 0.13^{bc}	0.73 ± 0.07^{d}	27.5 ± 8.36^{a}	14.2 ± 2.15^{cde}	3.83 ± 0.41^{ab}
16-2-4	1.50 ± 0.1^{cde}	0.85 ± 0.12^{ab}	24 ± 5.63^{abc}	16.57 ± 2.14^{ab}	3.24 ± 0.09^{de}
16-2-6	1.05 ± 0.13^{fg}	0.73 ± 0.04^{cd}	20.73 ± 915^{abcd}	14.1 ± 1.35^{cde}	3.08 ± 0.08^{e}
16-2-8	1.49 ± 0.1^{cde}	0.71 ± 0.03^{d}	26.35 ± 3.2^{ab}	13.42 ± 0.28^{e}	3.25 ± 0.07^{cde}
16-2-9	1.02 ± 0.03^{fg}	0.77 ± 0.08^{abcd}	15.9 ± 0.56^{cde}	17.37 ± 0.67^{a}	3.30 ± 0.08^{cde}
16-2-10	1.80 ± 0.12^{ab}	0.88 ± 0.07^{a}	19.23 ± 3.09^{abcd}	14.8 ± 1.35^{bcde}	3.37 ± 0.22^{cde}
16-2-11	1.53 ± 0.35^{cd}	0.71 ± 0.03^{d}	24.67 ± 5.59^{abc}	14.44 ± 0.86^{bcde}	3.09 ± 0.04^{e}
16-2-12	1.25 ± 0.02^{ef}	0.73 ± 0.04^{cd}	24.07 ± 2.71^{abc}	14.1 ± 0.30^{cde}	3.27 ± 0.09^{cde}
16-2-13	1.44 ± 0.03^{cde}	0.73 ± 0.09^{d}	28.27 ± 1.12^{a}	14.67 ± 0.45^{bcde}	3.26 ± 0.13^{cde}
16-2-14	1.82 ± 0.13^{ab}	0.73 ± 0.07^{d}	16.23 ± 1.72^{cde}	16.1 ± 2.11^{abcd}	3.25 ± 0.32^{cde}
16-2-15	1.88 ± 0.06^{ab}	0.71 ± 0.03^{d}	16.9 ± 3.42^{cde}	14.38 ± 1.53^{bcde}	3.39 ± 0.33^{cde}
16-2-16	1.66 ± 0.06^{bc}	0.73 ± 0.05^{cd}	21.19 ± 5.73^{abcd}	14.39 ± 0.93^{bcde}	3.57 ± 0.21^{bcd}
16-2-17	1.37 ± 0.03^{de}	0.78 ± 0.04^{abcd}	17.27 ± 4.13^{bcd}	17.13 ± 1.03^{a}	3.99 ± 0.08^{a}
16-2-22	1.38 ± 0.02^{de}	0.77 ± 0.07^{bcd}	24.4 ± 8.61^{abc}	16.2 ± 1.04^{abc}	3.82 ± 0.37^{ab}
16-2-24	1.68 ± 0.02^{bc}	0.75 ± 0.02^{bcd}	18.23 ± 6.16	14.43 ± 0.9^{bcde}	3.63 ± 0.19^{abcd}
16-2-25	1.81 ± 0.02^{ab}	0.76 ± 0.03^{bcd}	8.13 ± 1.51^{e}	12.87 ± 1.69^{e}	3.59 ± 0.53^{abcd}
16-2-26	2.05 ± 0.05^{a}	0.77 ± 0.02^{abcd}	16.7 ± 0.62	13.8 ± 0.69^{de}	3.68 ± 0.07^{abc}
16-2-31	1.33 ± 0.1^{de}	0.76 ± 0.04^{bcd}	13.17 ± 1.5	14.4 ± 0.46^{bcde}	3.40 ± 0.11^{bcde}
Simultan	1.93 ± 0.17^{de}	0.83 ± 0.06^{abc}	30.22 ± 7.58^{a}	17.63 ± 6.56^{a}	3.47 ± 0.43^{bcde}
Northblue	2.04 ± 0.29^{a}	0.78 ± 0.04^{abcd}	15.33 ± 5.38^{cde}	13.14 ± 2.76^{e}	3.23 ± 0.32^{de}

*Means with the same letter are not significantly different at 5% level

Table 2. The average values of total content of polyphenols, flavonoids, antocyannins and total sugar content at 22 blueberry genotypes

Genotypes	TPC (mg GAE/ 100 g)	TFC (mg CE/100 g)	TAC (C 3 - G mg/100 g)	TSC (%)
16-2-1	581.33±3.06^{cdef*}	182.67±0.58^{bc}	316.15±89.67^{fgh}	11.56±0.01^{c}
16-2-2	399.67±16.07^{f}	124.33±4.51^{defgh}	222.07±15.84^{gh}	6.38±0.02^{gh}
16-2-3	549.67±15.5^{cdef}	88.67±15.04^{hi}	368.08±13.79^{efg}	4.76±0.05^{j}
16-2-4	615.33±22.37^{cdef}	196.33±0.58^{ab}	274.33±6.66^{gh}	8.61±0.01^{e}
16-2-6	414.33±0.58^{ef}	134±0.41^{defg}	575±10^{bc}	6.49±0.01^{g}
16-2-8	582.33±74.9^{cdef}	139.00±3.21^{de}	280.67±7.09^{fgh}	6.42±0.01^{gh}
16-2-9	389±13.45^{f}	138.00±0.2^{def}	834.00±89.71^{a}	5.59±0.3^{hi}
16-2-10	401±27.4^{f}	108.67±2.52^{efghi}	263.82±10.42^{gh}	5.01±0.01^{j}
16-2-11	464.67±59.08^{def}	195.67±5.69^{ab}	529.02±22.06^{cd}	4.86±0.02^{j}
16-2-12	954.33±103.85^{b}	101.67±3.06^{fghi}	472.97±42.03^{cde}	6.84±0.03^{g}
16-2-13	145.67±4.51^{g}	113.67±3.06^{efghi}	163.83±6.5^{h}	6.77±0.02
16-2-14	657±12,12^{bcd}	87.00±0.21^{i}	348±140.58^{efg}	12.67±0.58^{b}
16-2-15	709.33±22.37^{cd}	136.33±7.57^{def}	474.92±32.09^{cde}	5.9±0.03^{hi}
16-2-16	969±296.08^{b}	151.83±51.05^{cd}	427.56±199.97^{def}	5.4±0.62^{ij}
16-2-17	651±0.1^{bcd}	221.00±0.32^{a}	686.69±0.09^{b}	8.04±0.01^{f}
16-2-22	1296.67±0.58^{a}	118.67±0.58^{defghi}	269.28±0.07^{gh}	7.73±0.01
16-2-24	639±0.1^{bcdef}	203.67±0.58^{ab}	162.96±0.05^{h}	13.28±0.01^{a}
16-2-25	1202.33±0.58^{a}	129±0.9^{defg}	177.22±0.12^{h}	10.05±0.01^{d}
16-2-26	610±0.1^{cdef}	118.00±29.44^{defghi}	355.55±0.05^{efg}	10.29±0.03^{d}
16-2-31	793.67±354.49^{bc}	97.47±15.61^{ghi}	297.94±0.10^{fgh}	10.40±1.03^{d}
Simultan	668.44±109.43^{bcd}	195.04±57.15^{ab}	514.41±236.47^{cd}	7.40±3.04^{f}
Northblue	505.66±181.66^{def}	211.39±79.73^{a}	389.77±222.06^{efg}	8.09±2.96^{f}

[*]Means with the same letter are not significantly different at 5% leve.

The biggest level of total anthocyanins pigments (TAC) 834.00 C 3 - G mg 100 g recorded by the hybrid 16-2-9, followed by 16-2-17 (686.69 C 3 - G mg 100 g). The genitors had the lower level for 'Northblue' cv. (389.77 C 3 - G mg 100 g). Regarding the total vitamin C content (Table 3) in blueberry fruits of 'Simultan' and 'Northblue' cultivars and their progenies hybrids, oscillated between 4.76 mg/100 g (16-2-31 hybrid), and 12.67 mg/100 g (16-2-1 hybrid). In this case, genitors contained lower vitamin C compared to most of their hybrids. Carotenoids (Car) are bioactive substances in foods with powerful antioxidant bioactivity. There are abundant data showing theirs preventive effects in humans for a number of diseases (Stahl & Sies, 2003; Lila, 2004; Schmidt, 2004). Carotenoids include diverse compounds such as lycopene, α- and β-carotene. The lycopene level oscillated between 0.01 mg/100 g (16-2-3 hybrid) and 0.37 mg/100 g (16-2-1 hybrid). Also, the β-carotene level varied from 0.02 mg/100 g (16-2-3 hybrid) to 0.42 mg/100 g (16-2-1 hybrid).

Table 3. The average values of total content of Vitamin C, β-carotene and lycopene at 22 blueberry genotypes

Genotypes	Vitamin C (mg/100g)	β-carotene (mg/100g)	Lycopene (mg/100g)
16-2-1	12.47±0.01^{abc}	0.42±0.04^{a}	0.37±0.03^{a}
16-2-2	12.07±0.07^{abcd}	0.07±0.04^{cde}	0.06±0.1^{fg}
16-2-3	7.21±0.16^{abcdefg}	0.02±0.02^{e}	0.01±0.06^{h}
16-2-4	7.42±0.02^{abcdefg}	0.09±0.01^{cde}	0.06±0.07^{fg}
16-2-6	12.90±0.02^{ab}	0.22±0.02^{b}	0.2±0.01^{b}
16-2-8	11.73±0.36^{abcdef}	0.13±0.01^{c}	0.09±0.01^{def}
16-2-9	11.35±0.01^{abcde}	0.07±0.01^{cde}	0.1±0.01^{cd}
16-2-10	10.23±0.13^{abcdef}	0.07±0.01^{cde}	0.06±0.02^{fg}
16-2-11	11.34±0.29 ^{abcdef}	0.1±0.01^{cd}	0.06±0.01^{fg}
16-2-12	13.49±0.51^{a}	0.03±0.01^{de}	0.03±0.02^{fg}
16-2-13	8.86±0.01 ^{abcdefg}	0.07±0.02^{cde}	0.06±0.01^{fg}
16-2-14	3.77±0^{g}	0.2±0.01^{b}	0.12±0.01
16-2-15	7.21±0.11 ^{abcdefg}	0.08±0.01^{cde}	0.06±0.01^{g}
16-2-16	7.42±1.59^{abcdefg}	0.11±0.09^{cd}	0.09±0.03
16-2-17	4.91±0.01^{fg}	0.04±0.01^{de}	0.03±0.01^{h}
16-2-22	5.12±0.02^{efg}	0.13±0.01^{c}	0.07±0.01^{efg}
16-2-24	5.96±0.01^{cdefg}	0.11±0.01^{cd}	0.06±0.01^{fg}
16-2-25	3.89±0.01^{g}	0.06±0.01^{cde}	0.07±0
16-2-26	4.79±0.54^{fg}	0.11±0.12^{cd}	0.08±0.04
16-2-31	3.48±0.16^{g}	0.02±0.03^{e}	0.02±0.01^{h}
Simultan	7.52±7.16^{abcdefg}	0.39±0.06^{a}	0.18±0.08^{b}
Northblue	6.29±6.8^{bcdefg}	0.64±0.45^{cde}	1.34±1.45^{c}

CONCLUSIONS

According to the results presented in our study, the berry weight, morphological and biochemical characteristics of the fruits of these new hybrids obtained by artificial hybridization were closely of the genitors or the progenies even exceeded their parents.

Important indices of biochemical evaluation, can be analyzed in the next years, in order to increase the efficiency of creating new cultivar. Our results will strengthen the breeding process in the Research Institute for Fruit Growing Pitesti which aims to create new valuable varieties of *Vaccinium corymbosum* L. with a significant complex of biologically active compounds, which makes the fruits of this crop a trendy food product

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RESEARCH REGARDING INTEGRATED PEST MANAGEMENT STRATEGIES IN SWEET CHERRY ORCHARDS IN SOUTH-EAST ROMANIA

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Abstract

Integrated Pest Management refers to a control system of harmful organisms designed to help farmers fight pests effectively by choosing the most appropriate technological, physical, chemical and biological practices to ensure a high yield, in terms of quantity and quality, and to be friendly to the environment and human health. Consequently, the European Union requires the application of several principles of Integrated Pest Management that fit within sustainable farm management. In sweet cherry orchards the combination of non-chemical methods that may be individually less efficient than pesticides can generate valuable synergies. In Dobroudja region, Constanta county, in sweet cherry orchards, it was successfully managed to integrate appropriate practices that discouraged the development of pests and limited the number of phytosanitary treatments to the minimum necessary level. A high level of yield was obtained to varieties Kordia, Van and Skeena, assessed in 2022.

***Key words**: sweet cherry, integrated pest management, harmful organisms, control*

INTRODUCTION

Due to public awareness of the health advantages of cherries, which are abundant in polyphenolics (namely anthocyanins and hydroxycinnamic acids), production and consumption of cherries have surged in recent years. Turkey, the USA, and Iran were the top producers of sweet cherries in the world, increasing production from 1.9 to 2.32 million tons over the past 16 years.

Thanks to their luxurious tasting fruits and the fact that they are among the first fruits to be found on the market, cherries are highly appreciated by consumers. They have high content in vitamins, mineral salts, sugars, too.

The priorities for sweet cherry farmers are increased fruit quality, such as size, appearance, firmness, flavor, and shelf life, ripening time and extending seasonality, self-fertility, abiotic and biotic stress resistance (Budan & Petre, 2006; Sansavini & Lugli, 2008). Also, a big challenge for farmers is to obtain fruit free of pesticide residues since year after year diseases and pests damage crops and large imports from neighbouring countries reach the domestic market. So that, in fruit growing, it is inconceivable to obtain high yields in terms of quantity and quality without the use of fertilisers and fungicides, as fruit trees occupied land is generally poorly fertile, terraced or unsuitable for other crops for a long time (Jalobă et al., 2019). In cherry, monilliosis in wet springs causes 60-100% loss of the fruit crop and makes the trees sick until they die. (Tomșa & Tomșa, 2003). Other deleterious diseases are bacterial spot (*Pseudomonas syringae* van Hall), shot-hole (*Stigmina carpophila* Léveillé, M.B. Ellis), anthracnose (*Coccomyces hiemalis* Higgins), leaf red spot (*Mycosphaerella cerasella* Aderhold), witches' broom (*Taphrina cerasi* Fuckel, Sadebeck), leaf scorch (*Gnomonia erythrostoma* Auerswald) and others.

As regards for pests the most damaging to cherry trees in Romania are the European cherry fly (*Rhagoletis cerasi* L.), San José scale (*Comstockaspis perniciosa* syn. *Quadraspidiotus perniciosus* C.), plum fruit moth (*Grapholita funebrana*, T.), apple and thorn fruit weevil (*Rhynchites aequatus* L., Coleoptera: Attelabidae), black cherry aphid (*Myzus cerasi*

F., Hemiptera: Aphididae), spotted wing drosophila (*Drosophila suzukii* M., Diptera: Drosophilidae).

Nowadays, buyers of sweet cherries and other stone fruits are rightfully worried about the contamination of the fruit with pesticides. Consequently, the requirement for naturally `clean and safe' agriculture products is still increasing (Pekar, 1999).

Pesticides with a focus on contact or systemic effects have historically been used to treat cherry diseases and pests from early spring until just before harvest. However, it is also desirable to minimize the use of plant protection products and their risk by 50% to fulfill the objectives of the "farm-to-fork" strategy (https://ec.europa.eu/food/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf). The EU Farm to Fork Strategy was introduced by the European Commission in May 2020 with the goal of ensuring a sustainable food value chain. The idea of Integrated Pest and Disease Management (IPM) has undergone more than 50 years of development and has been described and reduced numerous times. The IOBC (International Organization for Biological and Integrated Control of noxious animals and plants) is one company that has received special distinction for developing IPM tactics. The Sustainable Use Directive (directive 2009/128/EC), has made IPM the norm for crop protection in Europe. This study tested practical cultural control strategies for integration into traditional commercial sweet cherry growing, aimed at controlling specific pests and diseases and lessening the impact of pesticide use on cherry ecosystems and the environment.

MATERIALS AND METHODS

The research was conducted in a commercial sweet cherry orchard belonging to Ostrovit S.A., Constanța county in 2022, where the experimental plants material consisted of sweet cherry trees of the cultivar Kordia (row spacing: 4.5 m; plant spacing within the row: 2.5 m, vase-shaped crown) grafted on Gisela 5 rootstock, Van (6 m x 4 m, vase-shaped crown) grafted on Mahaleb rootstock and Skeena (6 m x 2 m, fan-trained tree) grafted on Colt. Eight randomized points of assessment were chosen over the whole surface of each variety. During growing season one hundred shoots, one hundred flowers and one hundred leaves were analysed visualy and in the laboratory under the microscope. One hundred fruits were examined before and during harvesting to assess pests and diseases incidence. It was used the following formula: Incidence % $=\frac{(n*100)}{N}$, where n represents number of plant organs affected and N is number of organs assessed (Vlad, 2020).

For pests there were assessements, seasonal abundance was obtained by using pheromone traps and yellow double-sticky traps (10 traps/orchard), hung in the middle exterior tree canopy, 1.5-2 m above the ground, one trap per tree, at a distance of 10-13 m from each other and replaced every 5 days from April to June. Other attractants after 3-4 weeks. The ecological parameters of abundance (A), dominance (D%), constancy (C%) and ecological significance (W%) have been calculated using specific formulas (Simionescu, 1983; Stan, 1994; Baban, 2006): $D\% = \frac{A \times 100}{n}$, where: A - number of individuals in a species (abundance); n - total number of individuals of all species in a sample.

For the dominance values, species were classified as subreceding species (D1 < 1%), receding species (D2 = 1-2%), subdominant species (D3 = 2-5%), dominant species (D4 = 5-10%) and eudominant species (D5 > 10%).

$C\% = \frac{ns \times 100}{n}$, where: ns - number of samples with one species; n - total number of samples.

Regarding constancy values, species were classified as accidental species (C1 = 1-25%), accessory species (C2 = 25-50%), constant species (C3 = 50-75%), and euconstant species (C4 = 75-100%).

$W\% = \frac{D \times C}{100}$ For the ecological significance, species were classified as accidental species (W1 < 1%), accessory species (W2 = 1-5%) and characteristic species (W3 > 5%).

IPM measures

Organizational measures. When orchard was set up, the planting material held a phytosanitary passport and was free of viroids. The varieties settled are resistant or diseases - tolerant and yield efficient since the rootstocks are very suitable to Dobroudja soil conditions (especially Colt).

The exposure of the orchard is east and south – east and is terraced on a hill bounded by a protective hedge formed of acacia and carob trees. It is located near a branch of the Danube in the Pacuiul lui Soare area.

Trees were designed by yearly pruning as vase-shaped form or fan-shaped. This improve the structure, increase flowering wood and assist with disease resistance by having good air flow and low humidity. Planting density is wide enough to reduce diseases (humidity), correct spacing (no damage - entrance for bacteria). Orchard soil has good drainage that increases benefitial insects and decrease bacterial infections.

Hygenic measures for machinery, equipments and tools are taken so that at the end of every working day they are cleaned or disinfected.

Spraying atomizers calibration is executed by an authorized professional team that inspects and adjust every year. The spraying machines uses a drift-reducing technology.

Storage of pesticides and PPP remnants are managed in accordance with EU laws and the disposal of empty packages follows the national programme SCAPA (The Pesticide Packaging Collection System).

All the fruits are harvested in the whole orchard so as not to keep a source of infection for the next season.

Cultivation techiniques. Mechanical control of the specific weeds orchards is practiced by mowing between rows and by hoeing between trees in order to remove them because they may be vector viruses and serve as alternate hosts for harmful insects. Also, the water in the soil is thus preserved by the vegetal cover.

The branches and plant material resulting from pruning are ground by a prunings harvesting chopper (Figure 1). This enhaces the fertility of the soil.

Figure 1. Chopped prunings

Removal of infected plant parts and prepruning are carried out by qualified workers.

If frosts occurs between buds burst stage and fruit set stage (especially at flowering), during the night, orchard is protected by biofumigation in order to save the yield.

Tree trunks are painted with lime in the spring to prevent certain pests to reach their damage place.

Organic fertilization (18 t/ha) is provided by livestock manure from the company's Angus cow farm located in the orchard located nearby.

Irrigation and treatments water is assured by water pumped from the neighbouring Danube.

During flowering, over-pollination with Tripol bumblebees (*Bumbus terestris*) hives is used (Figure 2).

Figure 2. Pollination hives

Monitoring. In this case study two types of traps were used for pest monitoring, pheromone traps and yellow double sticky traps with the dimensions of 20 x 40 cm (Figure 3).

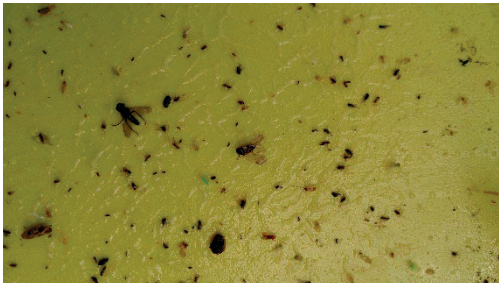

Figure 3. Yellow sticky trap

These traps were checked out daily by specialized personnel and the results were recorded on notebooks and reported to the central data organizer.

Treatments. Spraying schedule (Table 1)

Table 1. Treatments applied in cherry orchard

Phenophase	Plant protection Product	Target
Winter dormancy	Bouillie Bordelaise WDG 5 kg/ha Polisulf Tip MIF 20l/ha	Bacterial and fungal diseases, common red spider mite, San José scale
Before bloom	Merpan 80 WP 2 kg/ha Vitra 50 WP 1.5 kg/ha Teppeki 0.140 l/ha Evobor	Monilinia, leaf spot, shoot blight leaf scorch, leaf spot, crown gall, aphids, flowering, stress resistance
During bloom	Score 250 EC 0.2l/ha Navu Forte 4 kg/ha Algamin 2.5 l/ha Kelom EDTA Calcium 1 kg/ha	Monilinia, leaf spot, shoot blight, foliar fertilization, resistance, colour, growth
After bloom	Signum 0.5 kg/ha Exirel 0.75 l/ha Fertigofol Ultra 4l/ha Borocal 2l/ha	Monilinia, anthracnose, cherry fruit fly, fertilization, fruit fall, firmness
Fruit about half final size	Dodifun SC 1.25 l/ha Chorus 50 0.6 kg/ha Karate Zeon 0.15l/ha Cropmax 2kg/ha Xilato ZnMn 1l/ha Rizammina 2.0 kg/ha	Leaf spot, monilinia, cherry fruit fly, fertilization, foliar fertilizer
Fruit about 70% of final size	Switch 1 kg/ha Merpan 80 WP 2 kg/ha Movento 100 SC 1.8 l/ha Algamin 2.5 l/ha	Monilinia, shoot blight fruit fly, aphids resistance, colour

RESULTS AND DISCUSSIONS

During the spring at flowering stage, weather conditions made it necessary to carry out two fumigation interventions during the night due to the drop in temperature down to -3 degrees. However, the percentage of affected flowers was insignificant (3%). The flowers continued to develop and additional pollination resulted in a higher number of tied fruits. Treatments scheme applied in the orchard together with IPM measures proved very good efficacy in diseases management. Their incidence % is shown in Table 2.

Table 2. Diseases incidence % in cherry varieties

Pathogen	Kordia	Van	Skeena
Viroids	0	0	0
Pseudomonas syringae	0	0	0
Taphrina cerasi	0	0	0
Stigmina carpophila	8	5	6
Gnomonia erythrostoma	1	2	1
Monilinia laxa (shoots)	2	1	1
Monilinia laxa (flowers)	3	5	2
Monilinia fructigena (fruits)	7	3	5
Coccomyces hiemalis (leaves)	10	5	5
Nectria species	0	0	0

Moniliosis of cherry was very well controlled so as it had a very low incidence: 1-2% on shoots, 2-5% on flowers and 3-7% on fruits. Most common disease was anthracnose which reached a maximum incidence of 10% in Kordia variety. Symptoms of other diseases didn`t occur or they were very weak.

Three mechanised interventions have provided good weed control, but the biggest problem is *Sorghum halepense*, an invasive perennial weed that can regenerate very quickly after mowing, especially after high amount of rainfall. Out of a total of 117 traps the total number of individuals was 455 of which the dominant species were *Rhagoletis cerasi*, *Grapholita funebrana*, *Anarsia lineatella*, *Rhynchites aequatus* (Table 3). A relatively low abundance had also the species *Q. perniciosus* and *M. cerasi*, which do not appear in the table, but because pest control is difficult, the situation needs to be regularly monitored.

Table 3. Cherry pests captures on yellow double-sticky traps in the whole orchard

Taxa	Sweet cherry						
	A	D		C		W	
	(no)	Class	%	Class	%	Class	%
Tephritidae							
Rhagoletis cerasi, Linnaeus, 1758	45	9.89	D4	51.28	C3	5.07	W3
Tortricidae							
Grapholita funebrana, Treitschke, 1835	30	6.59	D4	34.18	C2	2.25	W2
Gelechiidae							
Anarsia lineatella, Zeller, 1839	10	2.19	D3	17.09	C1	0.37	W1
Attelabidae							
Rhynchites aequatus, Schneider, 1791	15	3.29	D3	8.54	C1	0.28	W1

Maintaining a clean orchard by picking up dropped fruit from the orchard floor and removing wild and abandoned host trees are two crucial cultural controls that growers can adopt to prevent or reduce European cherry fruit fly (*R. cerasi*) outbreaks. Adding ground cover, weed barrier cloth, or mulch to the soil beneath the tree canopy will also aid in preventing larvae from burrowing into the soil

or emerging adults from appearing from the soil.
After the end of the cherries harvesting in the whole orchard, yields were calculated and reported. The following productions resulted: Kordia 13.8 t/ha; Skeena 11.5 t/ha; Van 12.4 t/ha. Fruits were very good - looking with excellent marketability (Figure 4). Cracking index was at a low level for each variety, ranging from 2 to 5%.

Figure 4. Van harvested fruits

CONCLUSIONS

In the Ostrov fruit-growing area, sweet cherry has been grown for a long time, so there is a high load of pathogenic inoculum. The most dangerous pathogen, monilliosis, was successfully controlled, creating the conditions for a high yield, commercial appearance and increased marketability. Other cherry diseases have not caused any problems, but they should be monitored in the future. Leaf blight of cherry (anthracnose) had the highest level of incidence (10% at Kordia variety), but this fact didn`t endangered the quantity and quality of the yield.
Besides targeted pest control and optimisation of the way in which chemical treatments are applied, one can start to think about diversification in and around the cherry fields to reduce pest pressure or increase biological control. Also, this can be adjusted to improve the crop's capacity to withstand pests, be less visible or attractive or be more competitive.
Climatic factors such as temperature, wind, rain, and relative humidity, as well as natural enemies, play important roles in the population dynamics. Any of the regional pest species may experience an outbreak in good years and environmental circumstances. With a focus on cost-effectiveness and environmental safety, sanitation and harvest management become important for every orchard. In order to mitigate the effects of disease and pests, our work highlights the necessity to investigate the potential impacts of local management in cherry orchards. However, a strong experimental design is still lacking to demonstrate the significance of the identified cultural practices. Given the unpredictability of insecticide use (E.U. wanting to reduce by 50% the use of chemical pesticides by 2030), local crop management cannot entirely rely on insecticide control.

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EVALUATION OF PEAR AUTOCHTONOUS GENETIC RESOURCES REGARDING BEHAVIOUR TO MAIN DISEASES AND PESTS UNDER FIELD CONDITIONS

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Abstract

In this paper we proposed to evaluate 53 pear genotypes collected in the germplasm collection of the Research Institute for Fruit Growing Pitesti, Romania regarding the behaviour to the main diseases and pests, in order to identify potential genitors for future breeding work. The evaluation was carried out between 2020 and 2022 after a scale from 1 (no symptoms) to 9 (very sensitive). The results obtained showed that there are enough sources of genes for resistance, as follows: `Cu miezul roşu`, `Anţig`, `Harbuzeşti`, `Para lui Niţă`, `Pere gutui`, `Tudor`, `Haydeea`, `Argessis`, `Romcor`, `Cristal`, `Paradise`, `Euras`, `Aniversare` cvs. for resistance/tolerance to fire blight; `Ervina`, `Romcor`, `Para lui Niţă`, `Cristal`, `Tudor`, `Mustoase`, `Paradise`, `Daciana`, `Carpica`, `Republica` cvs. for resistance/tolerance to pear scab;`Paradox`, `Corina`, `Pepenii`, `Haydeea`, `Argessis`, `Untoasă de Târgu Mureş`, `Isadora` - for the resistance/tolerance to Psylla. Some of these cultivars (`Cu miezul roşu`, `Isadora`, `Monica`, `Haydeea`, `Ervina`, `Cristal`, `Euras`, `Paradise`) have already been introduced into artificial hybridization carried out in the last years.

Key words: *breeding, Erwinia amylovora, Psylla sp., Pyrus, resistance, Venturia pyrina.*

INTRODUCTION

The pears are cultivated today in all continents, both in the northern hemisphere and in the southern hemisphere, totaling a production of over 25 metric million tons in 2021 (FAOSTAT, 2023). The genus *Pyrus* has at least 22 known species, all of which are native to Asia, Europe and the northern areas of the United States (Bell et al., 1996).

Disease and pests resistance is very important objective to most pear breeders. Numerous programs to improve the pears that are ongoing throughout the world have as main objective the increase in fire blight resistance (the eastern and southern parts of North America, and many regions of Europe), also to pears *Psylla* (*Cacopsylla pyricola* Foerster) and scab (*Venturia pirina* Aderhold). Along with fire blight, *Psylla* is responsible, in large part, for the decline of pear orchard (Alonso et. al., 2007). In Europe the most pear varieties are come from *Pyrus communis*, also, in Romania. The worldwide production of pears is dependent on relatively few cultivars, the reduction of the number of varieties that are planted, as a result of the demand of the market, causes progressive erosion in the genetic heritage of the pear varieties.

In our country, the pear breeding programs provides the creation of a valuable assortment adapted to the local climatic conditions, superior quality and resistance /tolerance to diseases and pests. The main aim of these research programs were the conservation and evaluation of pear genetic resources and the diversification of disease and pests resistance sources.

The fire blight caused by the bacteria *Erwinia amylovora* (Burrill) is the most dangerous disease of the pear culture, and, in Romania, it remains one of the limiting factors of the pear culture. The attack of these bacteria manifests itself on flowers, fruits, stems, especially young ladders, to which a typical symptom appears, bending in the form of a stick, on which the dried leaves remain hanged. Combating the fungus through phytosanitary treatments is expensive and does not always work, most of the time the infected plantations must be cleared.

Most varieties from *Pyrus communis* are sensitive to *Erwinia amylovora*, among them are very valuable, such as: 'Williams', 'Clapp's Favorite', 'Comice', 'Anjou', 'Aurora' (van der Zwet, 1982, Thibault, B., 1983). Less than 5% of the pear varieties have a resistance appreciated as moderate, among the moderately resistant varieties are Seckel and Kieffer Sedling (Bell, 1990). Some of Romanian local varieties, such as: 'Decana Krier', 'Galbene', 'Pere de iarnă', 'Pere gutui', 'Tămâioase de Călimănești' are potential sources of genes for resistance to fire blight (Cociu et al., 1999).

The pear scab caused by the fungus *Venturia pirina* Aderh with manifestations on leaves, stems and fruits, causes damage to pear production and depreciate these quality. On the attacked fruits, brown spots appear and possibly crap leather, they remain small or can deform, and therefore their market value decreases. Scab generally is not relevant in pear culture as it is in apple. In scab management the main goal is the reduction or prevention of primary infections in spring. If this has been successfully controlled, secondary infections will not be serious.

From the research carried out so far regarding the scab, it appears that none of the varieties of *Pyrus communis* species is immune to the attack caused by the pathogen *Venturia pirina* Aderh. Among the varieties with a good behavior in the scab attack are 'Williams', 'Conference', 'Dr. Jules Guyot' (Bell, 1990), 'Republica', 'Euras', 'Argessis', 'Maria Romana', etc. (Braniste et Andrieș, 1990; Cociu et al., 1999).

Psylla attacks in three ways: it spreads a mycoplasm that causes pears; it injects a toxin on the tissue of the tree as it feed sand produces honeydew while feeding. The attack of this insect can stunt, defoliate, and even kill trees. Because *Psylla* rapidly develops insecticides resistance, chemical control is difficult to do. It has already developed localized resistance to pyretroids all across its range. As such, it is very important to alternate between pesticide classes to prevent or slow resistance (D. Alston, 2007).

The varieties sensitive to the *Psylla* attack are mostly from *Pyrus communis*. *Psylla* resistance has been found in Asian species *P. betulaefolia, P. calleryana, P. fauriei, P. ussuriensis, P. bretschneideri, P. pyrifolia, P. pashia.* In Romania, researches on resistance to *Psylla*, carried out by Braniste (1980) and Andrieș (1990) showed that species *P. lindlezi*, *P.korchinski, P. salicifolia, P. serotina, P. syriaca, P. serulata* are resistant, along with some biotypes and varieties with ascendancy in *P. serotina.* Some Romanian local varieties 'Bulgărești', 'Tomnatice', 'Crăiese', 'Cantalupești', 'Pletoase', 'Popești' and 'Imperiale' have a good behavior at the *Psylla* attack (Militaru et al., 2010; Braniste N., 2000).

The main aims of this study were the evaluation of pear genetic resourceslocated in the germplasm collection at the Research Institute for Fruit Growing Pitesti-Maracineni, and identifying the resistant/tolerant of diseases and pests that would help the reproduction program to obtain resistance/tolerance of varieties to these diseases and pests. The environmental sustainability of pear production would increase with the breeding and development cultivars with durable resistance/tolerance disease and pests.

MATERIALS AND METHODS

The experiment was carried out in pear germplasm collection at the Research Institute for Fruit Growing Pitesti-Maracineni, Romania, from 2020 to 2022. The total number of genotypes studied was 53, 32 of them are local genotypes, 19 are breed Romanian varieties and 3 foreign varieties (Table 5). Within the study, the resistance/tolerance of these genotypes to *Erwinia amylovora*, *Venturia pirina* and *Psylla* sp. was carry out.

Assessment the genotypes to this disease and pest was made in natural condition, with the same currently treatments as in commercial field, uniformly applied to all the cultivars. In collection of Research Institute for Fruit GrowingPitesti- Maracineni each genotype was represented by two trees grafted on franc rootstock, planted on 3.4 m between rows and 2 m between trees on row. In the field, macroscopic observations of the symptoms expression were made according to ECPGR characterization and evaluation descriptors for pear genetic resources, from 1: no visible

symptom to 9: maximum infection, tree completely affected, nearly dead; maximum infection, tree completely affected, nearly all organs with symptoms (Tables 2, 3, 4).
The aim of this study was to investigate and to compare the resistance/tolerance to *Erwinia amylovora*, *Venturia pirina* and *Psylla* sp. for 50 Romanian cultivars (32 local varieties and 18 varieties bred) and 3 foreign varieties.

RESULTS AND DISCUSSIONS

The evaluation of different pears cultivars to fire blight, scab and *Psylla* response showed than the main factor in the development of these diseases and pests was the genotype and the meteorological conditions. Climatic data for the vegetation season (March-October) over the study period 2020-2022, registered at Research Institute for Fruit Growing Pitesti-Maracineni, are shown in Table 1.

Table 1. Meteorological data, registered during 2020-2022

	Year	Months							
		III	IV	V	VI	VII	VIII	IX	X
Temperature (°C)	2020	7,7	10,9	15,0	19,6	22,1	22,2	18,9	12,4
	2021	4,1	8,6	15,6	19,3	23,5	22,4	15,6	7,9
	2022	3,6	10,1	16,4	21,1	22,9	22,6	15,6	12,0
Humidity (%)	2020	62,9	47,7	64,4	71,0	63,2	61,0	62,6	82,4
	2021	64,6	64,8	65,1	73,3	61,4	61,3	67,4	79,9
	2022	65,9	74,7	72,9	75,2	70,3	77,6	84,0	82,6
Rainfall (mm)	2020	30,0	21,1	104,1	166,2	52,0	29,8	68,2	92,7
	2021	66,8	38,4	65,4	104,0	33,5	74,0	14,3	36,3
	2022	19,4	88,0	72,6	25,6	25,3	142,1	49,6	4.3

Evaluation of *Erwinia amylovora* attack. Based on the visual observations related to *Erwinia* attack, in the most resistant class we list: 'Isadora', 'Argessis', 'Euras', 'Romcor', 'Triumf', 'Monica' (score 1 - no visible symptom). The 'Ervina' variety presented the highest average of the attack average of 1.6%, showed severe fire blight symptoms only in 2022, when many branches were damaged (Figure 1). The symptoms were also registered at 'Williams', 'Packham's Triumph', 'Lucele', 'Untoasă de Feleac' cvs. (Table 6).
Evaluation of *Venturia pirina* attack. The results showed that there are enough sources genes for resistance/tolerance to *Venturia pirina*. Cultivars studied showed a very strong scab resistance, only one of them, 'Tomnatice', was identified as sensitive to the pathogen agent with the average of the three years equal to 2.3 (Figure 2).

Figure 1. Fire blight symptomson 'Ervina'cv.

Figure 2. Pear scab symptoms on 'Tomnatice' cv.

Evaluation of *Psylla* attack. The studied varieties showed different responses to this pest attack. During the three years of study the following cultivars have been heavily affected: 'Falcă roșie', 'Văratice' with score average 3.3 for these three years of study, 'Xenia' cv. (2.6), 'Williams' cv. (2.3), 'Galbene tămâioase' cv. (2.6). In some scientific papers, 'Beurre Bosc', 'Conference', 'Williams', 'Abatele Fetel', 'Beurre Hardy', 'Clap's Favorite', 'Passe Crassane' cvs. are considered susceptible (Campbell, 2002) which partial confirm our study.
Romanian varieties 'Isadora' and 'Euras' presented a strong resistance to *Psylla.* The score average for all study period was equal to 1, no symptoms appeared on leaves or fruit.

Table 2. Infection assessment scale to fire blight on branches (Lateur, 1999)

Score	Observation in the orchard	Visual rating estimation Incidence (%)
1	No visible symptom	0
2	One or very few small infections, detectable only on close scrutiny of the tree	[0-1]
3	Directly apparent infections without important consequences for the tree	[1-5]
4	X	X
5	Disease widespread over the branches, inducing the death or the ablation of a large part of the crown	± 25
6	X	X
7	Heavy infection; about half of the crown is badly affected with risk of ablation or death	± 50
8	X	X
9	Maximum infection, tree completely affected, nearly dead	> 90

X = intermediate rating

Table 3. Global assessment scale for scab infection on leaves and fruits (adapted from Lateur and Populer, 1996)

Score	Observation in the orchard	Visual rating estimation	
		Incidence (%)	Severity (%)
1	No visible symptom	0	
2	A few small scab spots are detectable on close scrutiny of the tree	≤ 1	
3	Scab immediately apparent, with lesions very thinly scattered over the tree	> [1-5]	-
4	X	X	-
5	Infection widespread over the tree, majority of leaves/fruits with at least one lesion	≥ 50	≤ 5
6	X	≥ 50	X
7	Heavy infection; multiple lesion or more large surfaces covered by scab on most leaves/fruits. Partial leaf fall; some fruits with skin cracks in scabbed lesions	≥ 50	± 25
8	X	≥ 50	X
9	Maximum infection, leaves black with scab often fallen; fruits black with scab	≥ 50	>75

X = intermediate rating

Table 4. *Psylla* sp. on leaves and fruits (adapted from Lateur, 1999)

Score	Observation in the orchard	Visual rating estimation Incidence (%)
1	No visible symptom	0
2	One or very few foci, detectable only on close scrutiny of the tree	[0-1]
3	Directly apparent foci without consequences for the tree	[1-5]
4	X	X
5	Number of foci widespread over the branches, inducing the curling of leaves	± 25
6	X	X
7	Heavy infection; about half of the leaves/fruits is badly	± 50
8	X	X
9	Maximum infection, tree completely affected, nearly all organs with symptoms	> 90

X = intermediate rating

Table 5. Origin of pear cultivars included in evaluation at RIGF Pitești-Mărăcineni

No	Variety	Origin
1	Aniversare	Doynned'hiver x Curé
2	Anțig	Local variety
3	Argessis	Napoca x Butirra precoce Morettini
4	Boierești	Local variety
5	Busuioace	Local variety
6	Carpica	Napoca x Butirra precoce Morettini
7	Codiță	Local variety
8	Corina	Passe Crassane x (B.c.*Pyrus serotina* x Olivier de Seres)x Decana de iarna
9	Cristal	(Rosior pietros x Decana de iarna) x Decana de iarna x Beurre Hardy
10	Cu miezul roșu	Local variety
11	Daciana	Napoca x Butirra precoce Morettini
12	DecanaKrier	Local variety
13	Ervina	(*Pyrusserotina* x Williams) x Napoca
14	Euras	(B.c.*Pyrus serotina* x Olivier de Seres) x Decana de iarna
15	Falcă roșie	Local variety
16	Fetița	Local variety
17	Galbene tămâioase	Local variety
18	Harbuzești	Local variety
19	Haydeea	Beurré Hardy x Beurré six
20	Imperiale	Local variety
21	Isadora	Haydeea x Tse Li (*Pyrusserotina*)
22	Lucele	Local variety
23	Lucii timpurii	Local variety
24	Monica	Santa Maria x Principe di Gonzaga
25	Mustoase	Local variety
26	Napoca	Dr. Jules Guyot x pollen mix (Clapp's favorite, Beurré, Clairgeau, Williams, Pastravioare, Zaharoase de vara)
27	Nina de Vișani	Local variety
28	Packham's Triumph (control)	Uvedalé's St. Germain x Williams' Bon Chrétien
29	Para de vin	Local variety
30	Para lui Niță	Local variety
31	Paradise	H 26-67-73 x Pastravioare
32	Paradox	Monica x Pastravioare
33	Paramis	Monica x Passe Crassane
34	Pepenii	Local variety
35	Pere de iarnă	Local variety
36	Pere gutui	Local variety
37	Piperate de toamnă	Local variety
38	Postatele	Local variety
39	Republica	Doyenne d'hiver x Madame Levavasseur
40	Romcor	Passe Crassane x (*Pyrus serotina* x Olivier de Serres) x Decana Comisiei
41	Roșii de iulie	Local variety
42	Sărsării	Local variety
43	Sântâliești	Local variety
44	Tămâioasă mică	Local variety
45	Tomnatice	Local variety
46	Triumf	Napoca x Beurré Giffard
47	Tudor	*(Pyrus serotina* x Decana de iarna) x Passe Crassane) x 30-40 Angers
48	Untoasă de Ardeal	Local variety
49	Untoasă de Feleac	Local variety
50	Untoasă de Târgu Mureș	Local variety
51	Văratice	Local variety
52	Williams	UK
53	Xenia	Triomphe de Vienne x Nicolae Krier

Table 6. Mean score of fire blight attack

No.	Variety	2020	2021	2022	Average
1	Aniversare	1	1	1	1.0
2	Anțig	1	1	1	1.0
3	Argessis	1	1	1	1.0
4	Boierești	1	1	1	1.0
5	Busuioace	1	1	1	1.0
6	Carpica	3	1	1	1.6
7	Codiță	1	1	1	1.0
8	Corina	1	1	1	1.0
9	Cristal	1	1	1	1.0
10	Cu miezul roșu	1	1	1	1.0
11	Daciana	2	1	1	1.3
12	Decana Krier	1	1	1	1.0
13	Ervina	1	1	3	1.6
14	Euras	1	1	1	1.0
15	Falcă roșie	1	1	1	1.0
16	Fetița	1	1	1	1.0
17	Galbene tămâioase	1	1	1	1.0
18	Harbuzești	1	1	1	1.0
19	Haydeea	1	1	1	1.0
20	Imperiale	1	1	1	1.0
21	Isadora	1	1	1	1.0
22	Lucele	2	1	1	1.3
23	Lucii timpurii	1	1	1	1.0
24	Monica	1	1	1	1.0
25	Mustoase	1	1	1	1.0
26	Napoca	1	1	1	1.0
27	Nina de Vișani	1	1	1	1.0
28	Packham's Triumph	2	2	1	1.6
29	Para de vin	1	1	1	1.0
30	Para lui Niță	1	1	1	1.0
31	Paradise	1	1	1	1.0
32	Paradox	1	1	1	1.0
33	Paramis	1	1	1	1.0
34	Pepenii	1	1	1	1.0
35	Pere de iarnă	1	1	1	1.0
36	Pere gutui	1	1	1	1.0
37	Piperate de toamnă	1	1	1	1.0
38	Postatele	1	1	1	1.0
39	Republica	1	1	1	1.0
40	Romcor	1	1	1	1.0
41	Roșii de iulie	1	1	1	1.0
42	Sărsării	1	1	1	1.0
43	Sântâliești	1	1	1	1.0
44	Tămâioasă mică	1	1	1	1.0
45	Tomnatice	1	1	1	1.0
46	Triumf	1	1	1	1.0
47	Tudor	1	1	1	1.0
48	Untoasă de Ardeal	2	1	1	1.3
49	Untoasă de Feleac	1	2	1	1.3
50	Untoasă de Târgu Mureș	1	1	1	1.0
51	Văratice	1	1	1	1.0
52	Williams	2	1	1	1.3
53	Xenia	1	1	1	1.0

Table 7. Mean score of pear scab attack

No.	Variety	2020	2021	2022	Average
1	Aniversare	1	1	1	1.0
2	Anțig	1	1	1	1.0
3	Argessis	1	1	1	1.0
4	Boierești	1	1	1	1.0
5	Busuioace	1	1	1	1.0
6	Carpica	1	1	1	1.0
7	Codiță	1	1	1	1.0
8	Corina	1	1	1	1.0
9	Cristal	1	1	1	1.0
10	Cu miezul roșu	1	1	1	1.0
11	Daciana	1	1	1	1.0
12	Decana Krier	1	1	1	1.0
13	Ervina	1	1	1	1.0
14	Euras	1	1	1	1.0
15	Falcă roșie	1	1	1	1.0
16	Fetița	1	1	1	1.0
17	Galbene tămâioase	1	1	1	1.0
18	Harbuzești	1	1	1	1.0
19	Haydeea	1	1	1	1.0
20	Imperiale	1	1	1	1.0
21	Isadora	1	1	1	1.0
22	Lucele	1	1	1	1.0
23	Lucii timpurii	1	1	1	1.0
24	Monica	1	1	1	1.0
25	Mustoase	1	1	1	1.0
26	Napoca	1	1	1	1.0
27	Nina de Vișani	1	1	1	1.0
28	Packham's Triumph	1	1	1	1.0
29	Para de vin	1	1	1	1.0
30	Para lui Niță	1	1	1	1.0
31	Paradise	1	1	1	1.0
32	Paradox	1	1	1	1.0
33	Paramis	1	1	1	1.0
34	Pepenii	1	1	1	1.0
35	Pere de iarnă	1	1	1	1.0
36	Pere gutui	1	1	1	1.0
37	Piperate de toamnă	1	1	1	1.0
38	Postatele	1	1	1	1.0
39	Republica	1	1	1	1.0
40	Romcor	1	1	1	1.0
41	Roșii de iulie	1	1	1	1.0
42	Sărsării	1	1	1	1.0
43	Sântâliești	1	1	1	1.0
44	Tămâioasămică	1	1	1	1.0
45	Tomnatice	1	5	1	2.3
46	Triumf	1	1	1	1.0
47	Tudor	1	1	1	1.0
48	Untoasă de Ardeal	1	1	1	1.0
49	Untoasă de Feleac	1	1	1	1.0
50	Untoasă de Târgu Mureș	1	1	1	1.0
51	Văratice	1	1	1	1.0
52	Williams	1	1	1	1.0
53	Xenia	1	1	1	1.0

Table 8. Mean score to *Psylla* attack

No.	Variety	2020	2021	2022	Average
1	Aniversare	3	1	2	2.0
2	Anțig	2	1	2	1.6
3	Argessis	3	3	2	2.6
4	Boierești	3	3	2	2.6
5	Busuioace	2	2	2	2.0
6	Carpica	3	2	2	2.3
7	Codiță	3	3	3	3.0
8	Corina	1	2	1	1.3
9	Cristal	2	1	2	1.6
10	Cu miezul roșu	2	2	1	1.6
11	Daciana	3	2	2	2.3
12	Decana Krier	1	1	1	1.0
13	Ervina	1	1	1	1.0
14	Euras	1	1	1	1.0
15	Falcă roșie	5	3	2	3.3
16	Fetița	3	1	1	1.3
17	Galbene tămâioase	3	3	2	2.6
18	Harbuzești	3	3	2	2.6
19	Haydeea	2	2	2	2.0
20	Imperiale	3	3	2	2.6
21	Isadora	1	1	1	1.0
22	Lucele	2	2	2	2.0
23	Lucii timpurii	3	3	1	2.3
24	Monica	1	1	2	1.3
25	Mustoase	3	3	3	3.0
26	Napoca	3	3	2	2.6
27	Nina de Vișani	3	3	2	2.6
28	Packham's Triumph	3	3	1	2.3
29	Para de vin	2	2	1	1.6
30	Para lui Niță	3	1	3	2.3
31	Paradise	3	1	1	1.6
32	Paradox	1	1	1	1.0
33	Paramis	2	2	2	2.0
34	Pepenii	1	1	1	1.0
35	Pere de iarnă	1	1	3	1.6
36	Pere gutui	2	2	2	2.0
37	Piperate de toamnă	3	2	2	2.3
38	Postatele	2	2	2	2.0
39	Republica	2	1	2	1.6
40	Romcor	2	1	1	1.3
41	Roșii de iulie	3	3	3	3.0
42	Sârsării	2	2	2	2.0
43	Sântăliești	3	2	1	2.0
44	Tămâioasă mică	3	2	1	2.0
45	Tomnatice	2	2	2	2.0
46	Triumf	2	3	2	2.3
47	Tudor	2	1	1	1.3
48	Untoasă de Ardeal	2	2	2	2.0
49	Untoasă de Feleac	3	2	2	2.3
50	Untoasă de Târgu Mureș	3	2	2	2.3
51	Văratice	5	3	2	3.3
52	Williams	3	2	2	2.3
53	Xenia	3	3	2	2.6

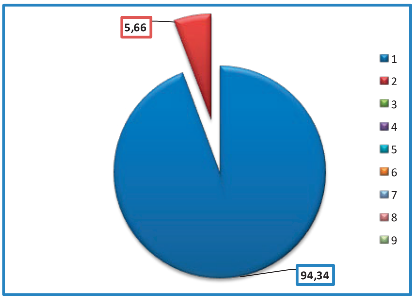

Figure 3. Proportion of genotypes with fire blight symptoms

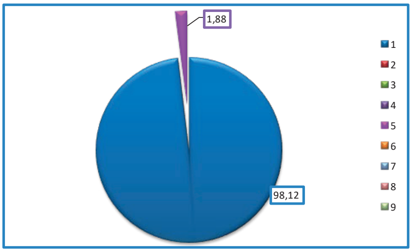

Figure 4. Proportion of genotypes with pear scab symptoms

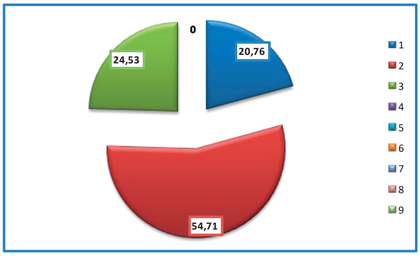

Figure 5. Proportion of genotypes with pear *Psylla* attack symptoms

CONCLUSIONS

The different response of pear varieties to diseases and pests attack, tested at RIFG Pitesti, Romania denoted a large variability, a strong influence of the genotype in the expression of resistance or tolerance to the *Erwina amylovora*, *Venturia pirina* and *Psylla* sp. Also, the meteorological conditions influenced the appearance of these pathogens and pests.

The Romanian cultivar 'Isadora' was noted for high resistance to diseases and pest compared to other cultivars tested in the same conditions.

For fire blight, 50 pear cultivars (94.34% of studied genotypes), were registered with “no visible symptom”.

From 53 studied genotypes, 'Tomnatice' was the scab susceptible cultivar, only, which is not recommended to be grown in climatic conditions from Mărăcineni, Argeș.

The response of the 53 genotypes studied to *Psylla* attack was very different: 20.76% of them with “no visible symptom”, 54.71% with “one or very few foci, detectable only on close scrutiny of the tree” and 24.53% was “directly apparent foci without consequences for the tree”.

Several cultivars registered with “no visible symptom” and considered as resistance/tolerance to diseases and pests could be used for further pear breeding programs.

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STUDY OF THE WATER REGIME IN SOME SWEET CHERRY CULTIVARS UNDER NORTH-EASTERN ROMANIAN CONDITIONS

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Abstract

The soil and climate conditions in the North-East area of Romania are considered suitable for sweet cherry cultivation, but sometimes problems may arise due to the intensification of global climate changes, mainly in terms of the water component. This study was conducted during the year 2022 using three sweet cherry tree cultivars as biological material: 'Van', 'Andreiaș' and 'Margonia' from Research Station for Fruit Growing (RSFG) Iași. The aim of this research was to evaluate physiological aspects of the water regime of the sweet cherry tree by determining the rate of dehydration, water content as well as the stomatal conductance of the leaves from different levels of the trees. Physiological determinations were carried out in three different phenological stages according to Biologische Bundesanstalt, Bundessortenamt and CHemical Industry (BBCH) scale: full flowering (BBCH 65), fruit growth (BBCH 75), and fruit ripening (BBCH 89) and were correlated with the registered climate data.

Key words*: dehydration rate, Prunus avium* L.*, stomatal conductance, water content.*

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is an important fruit tree species in Romania and occupies an area of 6,120 ha and an average production of 74,930 t/year (FAO, 2021) with a large expansion in the following years through new established plantations.

Production and survival of fruit trees in temperate zones depend on phenophases and time of growth in synchrony with temperature seasonal changes. Recent research shows that global climatic changes have influenced plants especially in the development of phenological stages (Ansari and Davarynejad, 2008; Balaci et al., 2008; Sîrbu et al., 2016). Many results showed that climate change influence greatly the plant's growth and its development. Meteorological factors restrict the biological cycle as well as the plant's productive potential (Slabu et al., 2012).

Water and temperature stresses are the main limiting factors in crop production worldwide (Shah et al., 2011; Ozherelieva & Lyakhova, 2021). Low water availability and abnormally high temperatures cause changes in plant metabolism and lead to water exchange violation in plants, at early phenological stages, transpiration and photosynthesis are affected, efficiency of water use increases, while senescence and fruit falling are stimulated (Jităreanu et al., 2009). Influenced by drought, plants experience cell dehydration and suffer from a considerable increase in the temperature of their tissues; all having a direct impact on photosynthesis (Toma et al., 2008) and an indirect impact on the entire metabolism. By changing the metabolism, the lack of water affects productivity, taste of fruits, and wood density (Ozherelieva & Lyakhova, 2021).

In plant species diversity, sweet cherry is a tree species whose leaves with a larger open stomatal pore area have a higher stomatal conductance and typically higher rates of photosynthetic CO_2 assimilation and water loss through transpiration (Franks & Farquhar, 2007). However, plants must maintain their hydration within narrow limits, and the high transpiration rate causes the water potential to decrease throughout the plant, which would cause mesophyll damage and xylem embolism during drought. Plants thus close their stomata in response to the drop in leaf water potential (McElwain, 2016; Henry et al., 2019).

The objective of this study was to evaluate the behaviour of some sweet cherry cultivars based on their phenological stages progress and on the dynamics of the water regime in terms of climatic conditions of the year 2022 in the North-East (N-E) area of Romania.

MATERIALS AND METHODS

The experiments were performed during 2022 on three sweet cherry cultivars existing in the germplasm fund from Research Station for Fruit Growing (RSFG) Iaşi - Romania. The studied sweet cherry cultivars: 'Van', 'Andreiaș' and 'Margonia' were cultivated on *P. mahaleb* L. seedlings rootstock.

The foliar dehydration rate was performed at 1, 2, 3, 4 and 24 hours intervals, determining the total content of bound water, referring to the percentage of water lost in the first hour and in 24 hours, depending also on the free water content of the samples. Water content of plant leaves was calculated by the formula:

$DR = \frac{x_{1-n}}{x_0} \times 100\%$, where: DR - foliar dehydration, %; x_0 - the first weighing of the leaf, g; x_{1-n} - leaf weight after certain periods of time (one to 24 hours), g (Jităreanu & Marta, 2020).

Dry matter content in leaves was determined, using the formula (Cupcea et al., 1965, cited by Popoviciu, 2018):

$DM = \frac{m_f}{m_i} \times 100\%$, where: DM - Dry matter content, %; m_i - weight of fresh material, g and m_f - weight of oven-dried material, g.

Stomatal conductance (g_s) measurements were carried out simultaneously with leaf water potential measurements, on the same trees by a leaf porometer. Leaf samples were taken from the sunexposed mature leaves of one year old shoots from different sides of the selected plants (Küçükyumuk et al., 2015).

Physiological determinations were performed on different phenological stages, according to Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale and Meier et al. (1994) at: full flowering (BBCH 65), fruit growth (BBCH 75) and fruit ripening (BBCH 89). The obtained results have been interpreted in relation with climatic conditions. The climatic data were recorded with the AgroExpert system of the RSFG Iaşi, Romania. Some obtained results were statistically analysed and the differences were determined by Duncan's test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

The evolution of climatic factors under the conditions of the year 2022, in the Iași area (North-Eastern of Romania) was analyzed every month and presented in Table 1.

Table 1. Climate condition at Iași county during the experimental period (RSFG Iași-Romania, 2022)

Month	Average annual temperature (°C)			Total annual precipitation (mm)		
	Monthly av.	Multiannual	Deviation	Monthly sum	Multiannual	Deviation
I	0.40	-3.30	-3.70	6.60	34.40	27.80
II	3.66	-1.50	-5.16	10.40	34.60	24.20
III	3.23	3.10	-0.13	56.94	28.90	-28.04
IV	10.02	10.30	0.28	58.00	28.90	-29.10
V	16.62	16.10	-0.52	17.40	27.40	10.00
VI	21.90	19.40	-2.50	26.60	28.10	1.50
VII	23.20	21.30	-1.90	27.80	40.30	12.50
VIII	22.50	20.50	-2.00	69.00	52.50	-16.50
IX	15.50	16.30	0.80	69.60	75.10	5.50
X	12.30	10.10	-2.20	12.60	69.20	56.60
XI	5.50	4.00	-1.50	69.20	57.60	-11.60
XII	1.40	-0.90	-2.30	16.20	40.80	24.60
Average/sum	**11.35**	**10.64**	**-0.71**	**440.34**	**517.80**	**77.46**

The monthly average of air temperature and the total amount of precipitation being established, as well as the deviation from the multiannual average. Thus, it was registered an annual average of 11.35°C, with a deviation from thermal multiannual average of 0.71°C.

The amount of precipitation recorded during analyzed period had values of 440.34 mm and was characterized by a deficit of 77.46 mm.

The months of interest for the pursuit of the studied phenophases were March, April, May, and June when the temperature was higher by +2.87°C than the multiannual average and precipitation deviation was higher with 45.64 mm although as annual average it was pedological drought. The absence of rain and the high diurnal and nocturnal temperatures lead to the appearance of pedological, atmospheric and physiological drought, thus shortening the phenophases (Jităreanu et al., 2009).

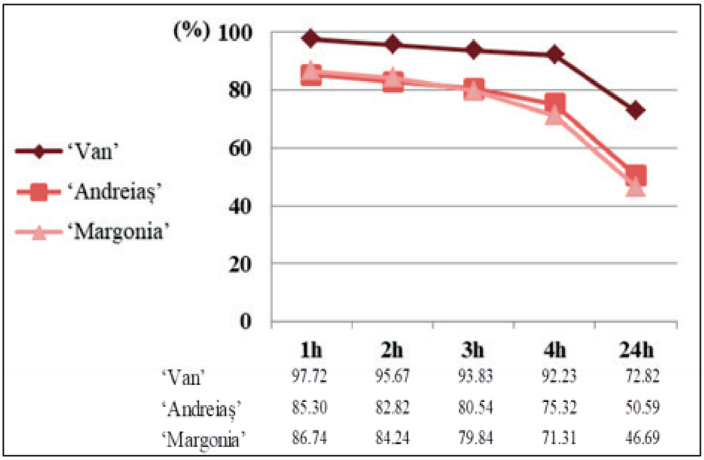

Figure 1. Evolution of dehydration rhythm in full flowering phenophase (65 BBCH sweet cherry cultivars, RSFG Iași, Romania, 2022)

The foliar dehydration rhythm was analyzed and graphically represented as follows: full flowering - 65 BBCH (Figure 1), fruit growth - 75 BBCH (Figure 2) and fruit ripening - 89 BBCH (Figure 3). In the flowering phenological stage, the dehydration rhythm drops from 97.72% ('Van'), 85.30% ('Andreiaș') and 86.74% ('Margonia') to 72.82%, 50.59% respectively, 46.69%, after 24 h. The most intense foliar rhythm of dehydration was recorded in the phenophase of fruit growth, recording differences of approximately 45% during 24 hours ('Van') (Figure 2). There are no evident differences between the studied cultivars.

During fruit ripening phenophase, the foliar dehydration rhythm was more intense at 'Andreiaș' cultivar, registering values of 31.67%.

The average values of the sweet cherry cultivars studied recorded minimum values of the dehydration rate, in the phenophase of fruit ripening, of 27.56% and maximum in the phenophase of fruit growth, of 39.24%.

These results are in a close relation with temperatures, normal for the period and with an excess of precipitation.

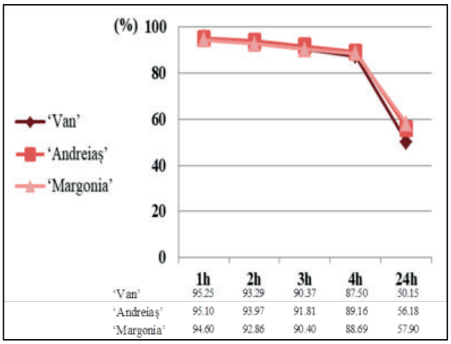

Figure 2. Evolution of dehydration rhythm in fruit growth phenophase (75 BBCH sweet cherry cultivars, RSFG Iași, Romania, 2022)

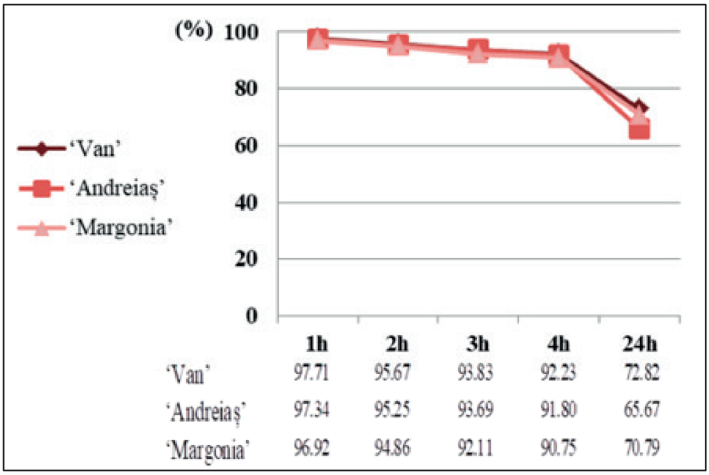

Figure 3. Evolution of dehydration rhythm in fruit ripening phenphase
(89 BBCH sweet cherry cultivars, RSFG Iași, Romania, 2022)

Averages of stomatal conductance measurements made in different phenological stages are shown graphically in Figure 4. The stomatal transpiration rate decreased during the fruit growth phenophase. Stomatal conductance recorded the highest values in the fruit ripening phenophase, between 14.73 ('Van') and 16.81 mol $H_2O/m^2/s$ ('Margonia').

The lowest stomatal conductance values were recorded in the phenophase of fruit growth, when all physiological processes are intensified and were between 7.01 ('Van') and 7.57 mol $H_2O/m^2/s$ ('Margonia').

The partial closure of stomata increases the availability of water in the plant and decreases dehydration stress in drought conditions (Henry et al., 2019). The varied stomatal conductance is a response of the trees to the water supply relative to the needs of the plant and the phenological stage (Downton et al., 1990).

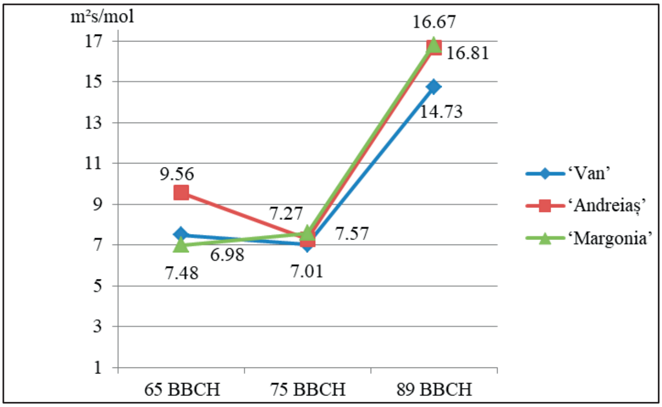

Figure 4. Stomatal conductance in different phenological stages
at sweet cherry cultivars (RSFG Iași, Romania, 2022)

The dry matter content of the leaves of the sweet cherry cultivars studied in three different phenological stages is presented in Table 2. The dry matter content values varied both between cultivars and phenophases. Thus, the highest dry substance content of the leaves was recorded in the phenophase of fruit ripening (36.22% at 'Van' cultivar, 37.91% 'Andreiaș' cultivar and 35.52% 'Margonia' cultivar). Statistically significant differences were highlighted between the flowering and fruit ripening phenophases. On average, the lowest amount of dry matter in the leaves was at 'Van' cultivar (29.09%) in flowering phenological stage. The maximum accumulation of dry matter in sweet cherry leaf tissues (36.55%) due the natural aging of the leaves was also accompanied by a decrease in water content, which is primarily due to environmental conditions.

Table 2. Dynamic of dry matter (%) content in the leaves in different phenophases at sweet cherry cultivars (RSFG Iași, Romania, 2022)

Phenophase[1]	'Van'[2]	'Andreiaș'	'Margonia'
65 BBCH	29.09[b]	33.40[b]	29.66[b]
75 BBCH	35.88[a]	30.81[b]	35.25[a]
89 BBCH	36.22[a]	37.91[a]	35.52[a]
Min.	**29.09**	**30.81**	**29.66**
Max.	**36.23**	**37.91**	**35.52**
Average	**33.74**	**34.05**	**33.48**

1-BBCH-Phenological growth stages (Meier et al., 1994): 65 (full fowering); 75 (fruit growth); 89 (fruit ripening);
2-Different letters after the number correspond with statistically significant differences for *p* 5% - Duncan test.

Other authors note an average of dry matter accumulation over the years, in drought summer conditions of 38.6%, 2.8% higher than in conditions of sufficient water availability, in the case of cherry (Ozherelieva & Lyakhova, 2021) and plum trees (Prudnikov, 2022).

The dry matter content is taken as a parameter associated with the optimal water regime and which characterizes the metabolic processes. The accumulation of dry matter by plants is the result of the relation with environmental factors and allow the assessment of growth and development conditions.

CONCLUSIONS

The climatic conditions analysed during the year 2022 had a favorable evolution in terms of temperature and the amount of precipitation for growth and development of the studied sweet cherry cultivars.

The most intense foliar dehydration rate was recorded in the phenophase of fruit growth and development, when in terms of total rainfall, a surplus was recorded.

The dry matter content of sweet cherry leaves varied according to climatic factors and the phenophase, being lower during periods with lower temperatures and abundant precipitation and higher when temperatures are higher and the precipitation quantitatively lower.

The optimal values regarding the sweet cherry studied cultivars water regime indicators have been in accordance with the phenological stage and climatic conditions.

The results of this study could help to improve the cultivation of sweet cherry, as well as other *Prunus* fruit trees with similar phenology and water stress behavior, not only in areas where water is insufficient, but also in regions where water availability is not a problem at the present, even without additional irrigation.

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THE EFFECT OF CLIMATIC ACCIDENTS ON PEACHES ÎN R.S.F.G. CONSTANȚA

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Abstract

In Dobrogea the culture of the peach tree has had great perspectives even from the beginning due to the extremely favorable climate. The peach species loves warm weather and has always found good growing and harvesting conditions in the south-eastern part of Romania. In this period, 7 Romanian and foreign peach tree cultivars were studied at Research Station for Fruit Growing (RSFG) Constanta: 'Mimi', 'Catherine sel.1', 'Springcrest', 'Raluca', 'Cardinal' 'Filip' and 'Cora'. This paper presents the manner in which certain peach tree cultivars reacted to frost in the springtime of 2020, 2021 and 2022, as well as the effect of the hail on Jun 14th, 2022 on the peach production. The greatest losses caused by the frost were registered in the spring-time of 2020 as far as the fructiferous buds are concerned: 90% at 'Springcrest', 80% at 'Cardinal', 60% at 'Raluca' and 'Filip', 40% at 'Mimi', 'Cora' and 'Catherine sel.1', 20%. The losses caused by the hail on July 14th, 2022 affected the production of the 'Springcrest' cultivar by 80% and of the 'Cardinal' and 'Cora' by 70%. The climatic changes that have been registered throughout the past 10 years have negatively influenced the culture of the peach tree and the effects have been classified according to the cultivar and its biology, as well as to the topographic placement of the allotments. The studies that have been carried out, together with the obtained results demonstrate the importance of choosing the cultivar assortment taking into account the favorability of the area, as well as the importance of installing anti-hail nets when setting up fruit-growing plantations

***Key words**: climate change, cultivar, hail, Prunus persica.*

INTRODUCTION

This paper deals with the manner in which the frost and the hail influenced the fruit production of certain peach tree and nectarine tree cultivars cultivated in Dobrogea in the years 2020, 2021 and 2022.

The frosts which occur in March and April after a relatively warm period are more dangerous than those which occur during the obligatory resting period (December-January). The floriferous buds in the pink button stage can resist to temperatures as low as -3.9°C for 2-3 hours; the blossomed flowers can tolerate a temperature of -2.8°C, while the newly tied fruit can resist to temperatures as low as -1.1°C (Chira et al., 2005).

The species of peaches and nectarines develop well in the climatic and soil conditions of Romania, especially in the area of Dobrogea (Dumitru, L.M,, 2003; Mihăilescu I. F.L. and Pavel M., 1993).

Previous research papers have revealed that the impact of climatic changes upon fruit-growing species can already be felt. For instance, by the end of the 1990s, the flowering of the trees in Germany was occurring several days earlier (Chmielewschi et al., 2004 and 2005). The vegetative season in Europe became longer by 10 days in the past 10 years (Chmielewschi and Rotzer, 2002). Due to the early flowering of the trees, in certain regions of Europe there was an increase in the risk of damage caused by late frosts (Anconelli et al., 2004; Sunley et al., 2006; Legave and Clazel, 2006; Legave et al., 2008; Chitu et al., 2004 and 2008) or by the disorders in the pollination and fruit setting processes (Zavalloni et al., 2006).

According to the estimations of the weather forecasts, there have been presented in the frame of the 4th report of the International Committee for Climatic Changes in the year 2007, the whole Europe and implicit Romania will be confronted in future with a process of global warming, characterized by increasing of temperatures with 0.5-1.5°C for the period

2020-2029 and with 2-5°C for the period 2029-2099. In the period 2090-2099 Romania will confront with pronounced drought during the time of summer. Researches from many countries, in the frame of climatic research methodology have the approached aspects regarding climatic changes effects on growth and development of some fruit tree species (Chmielewski and Rotzer et al., 2002; Olensen, 2002; Sunley et al., 2006; Chitu et al., 2010; Sumedrea et al, 2009). Climatic changes occurred also in Romania, they have determined meteorological phenomena, which are manifesting with augmented amplitude and intense frequency (severe drought, intense flooding, tornados, hail).

MATERIALS AND METHODS

The experimental plot is situated within the RSFG Constanța, with its headquarters in the village of Valu lui Traian, Constanța county, Dobrogea region, Romania. The geographical coordinates are: 44°10' North, 28°29' East, 70-72 m altitude.

During the period of 2020-2027 peach tree genotypes were studied, organised in a demonstrative plot that was created in 2011. The plot has 20 trees per row, with a planting distance of 4m × 4 m (625 trees/ha), with the canopy shape a vase and the rootstock a wild Tomis 1. Among the studied cultivars there, as well as cultivars promoted in the regional and national assortment, these are: 'Mimi', 'Catherine Sel.1', 'Springcrest', 'Raluca', 'Cardinal, 'Filip' and 'Cora'. The system used for the soil management system was with cultivated strips both between the rows as well as in the row. The soil is a calcareous chernozem (CZka), with a loamy texture and a high, alkaline pH (8.2) in its entire profile. All in all, the climatic conditions were favourable to the growth and fructification of the peach trees. The applied culture technology is the one specific to the peach tree: pruning phytosanitary treatments, soil works, irrigation, harvesting, conditioning and capitalisation of the fruit.

Due to climatic changes over the past few years, the resistance of peach trees seems to have become very different from one year to another. However, there are other factors involved as well, such as the topographic position of the orchard lot in which the peach trees are planted (in the case of the studied cultivars the land was the same - a plateau), the alternation between minimum and maximum temperatures during winter, which renders the trees less resistant and last but not least, the severity of climatic accidents.

The study focused on how certain peach tree cultivars reacted to hail damage and change in climatic conditions in the winters of the above-mentioned years.

In addition, the overall climatic conditions were favorable to the growth and fructification of the trees, with exception of the years 2020-2022, when a very strong frost was registered in both April and March, leading to the loss of some of the floriferous buds, while the hail on Jun 14th, 2022 affected the production of the Springcrest and Cora cultivars. With regard to these cultivars we observed the main fructification phenophases: the beginning of the blossoming, upon the appearance of the pink button; the beginning of the flowering, upon the appearance of the first open flowers; the ending of the flowering, when most of the flowers have lost their petals. The duration of the flowering phenophase at a certain cultivar can vary according to the action of the maximum temperatures during the day and the intensity of the wind, correlated with the degree of differentiation of the trees (i.e. the amount of flowers per tree). The intensity of the flowering was graded on scale from 0 to 5 - 0 being used when the cultivars displays no flowers at all, while 5 is used when the cultivar displays a plethora of flowers. The hardening of the core was determined by means of piercing it with a needle at regular intervals, usually 2 days. The process was carried out progressively and calendaristically, in the same day for all the observed cultivars. The harvesting maturity is largely influenced by a series of climatic and agro-technical factors, such as: temperature, drought, quantity of fruit per tree, shape of the head, density of the trees, etc. The observations and determinations were carried out 3-5 days after the climatic accidents recorded in 2020, 2021 and 2022, respectively and the production was assessed after the hail occurrence on July 11th, 2022. The hail, with a dimension of approximately 5-20 mm, seriously damaged the fruit production of some of the peach tree cultivars, more exactly those who had not been harvested until July 14th, 2022. The climatic data were recorded with the aid of an automatic meteorological station (the WatchDog type) and were processed as daily averages.

RESULTS AND DISCUSSION

In the period 2020-2022 the blossoming of the floriferous buds of the peach trees occurred between the following limits: between 09.03 and 16.03 for the 'Mimi' cultivar, between 18.03 and 27.03 at the 'Catherine Sel. 1' cultivar, between 13.03 and 27.03 at the 'Springcrest' cultivar, between 23.03 and 30.03 at the 'Raluca' cultivar, between 22.03 and 28.03 at the 'Filip, between 18.03 and 29.03 at the 'Cora' cultivar. Calendaristically the blossoming at the apricot tree occurred between 25.03 and 10.04 (16 days) in the studied years 2020-2022. (Table 1)

Table 1. The main stagers of fructfication and peach in the 2020-2022 period

No.	Cultivar	Year	The swelling of the floriferous buds	The flowering			Inten-sity	The hardeng of the stone	Harvesting maturity
				Begi-nning	Ending	Duration (days)			
1	Mimi	2020	11.03	24.03	08.04	15	5	04.06	29.07
		2021	09.03	22.03	04.04	14	5	10.06	27.07
		2022	16.03	25.03	10.04	16	5	09.06	22.07
		Limits ? / x	**09.03-16.03**	**18.03-24.03**	**08.04-14.04**	**14-16**	**5**	**04.06-10.06**	**22.07-29.07**
2	Catherine Sel. 1	2020	18.03	30.03	11.04	11	5	10.06	02.08
		2021	27.03	02.04	20.04	18	5	08.06	07.08
		2022	22.03	05.04	24.04	19	5	17.06	15.08
		Limits ? / x	**18.03-27.03**	**30.03-05.04**	**11.04-24.04**	**11-19**	**5**	**07.06-10.06**	**02.08-15.08**
3	Springcrest	2020	13.03	30.03	15.04	15	1	03.06	18.06
		2021	27.03	28.03	16.04	18	2	08.06	16.06
		2022	22.03	05.04	18.04	13	4	07.06	21.06
		Limits ? / x	**13.03-27.03**	**28.03-05.04**	**15.04-18.04**	**13-18**	**1-4**	**03.06-08.06**	**16.06-21.06**
4	Raluca	2020	26.03	04.04	17.04	13	2	06.06	18.07
		2021	30.03	09.04	23.04	14	3	10.06	20.07
		2022	23.03	20.04	28.04	8	4	08.06	24.07
		Limits ? / x	**23.03-30.03**	**04.04-20.04**	**17.04-28.04**	**8-14**	**2-4**	**06.06-10.06**	**18.07-24.07**
5	Cardinal	2020	22.03	05.04	20.04	15	2	07.06	15.07
		2021	28.03	11.04	18.04	7	3	10.06	13.07
		2022	23.03	20.04	30.04	10	3	08.06	18.07
		Limits ? / x	**22.03-28.03**	**05.04-20.04**	**18.04-30.04**	**7-15**	**2-3**	**07.06-10.06**	**13.07-18.07**
6	Filip	2020	22.03	09.04	19.04	10	4	07.06	17.07
		2021	28.03	03.04	12.04	9	5	09.06	20.07
		2022	25.03	05.04	12.04	7	5	10.06	22.07
		Limits ? / x	**22.03-28.03**	**03.04-09.04**	**12.04-19.04**	**7-10**	**4-5**	**07.06-10.06**	**17.07-22.07**
7	Cora	2020	18.03	01.04	10.04	10	5	07.06	20.06
		2021	29.03	06.04	17.04	11	4	09.06	27.06
		2022	25.03	18.04	27.04	9	5	10.06	25.06
		Limits ? / x	**18.03-29.03**	**01.04-18.04**	**10.04-27.04**	**9-11**	**4-5**	**07.06-10.06**	**20.06-27.06**

The beginning of the flowering. For all the studied cultivars the beginning of the flowering in the period 2020-2022 was recorded; however, the cultivars entered this phenophases at different times, albeit not necessarily significant (a few days from one cultivar to the next), so that mutual pollination was fully ensured. The limits for this phenophase were 18.03 and 20.04.

The ending of the flowering. In the studied period 2020-2022 the ending of the flowering occurred between 08.04 and 14.04 for the 'Mimi' cultivar, between 11.04 and 24.04 for the 'Catherine Sel. 1' cultivar, between 15.04 and 18.04 for the 'Springcrest' cultivar, between 17.04 and 28.04 for the 'Raluca' cultivar, between 18.04 and 30.04 for the 'Cardinal' cultivar, between 12.04 and 19.04 for the 'Filip' cultivar, between 10.04 and 27.04 for the 'Cora' cultivar. The dates were recorded as the days when the flowers lost their last petals. The duration of the flowering at the peach tree (average for the three studied years) expressed in number of days varied between 7 days (the 'Cardinal' cultivar in 2021) and 19 days (the 'Catherine Sel. 1' cultivar in 2022).

The intensity of the flowering. In 2020, 2021 and 2022 the following cultivars displayed a weak intensity of the flowering: 'Springcrest', 1 (2020), 'Auraş', 2 (2012), 'Springcrest', 2 (2021) and 'Raluca and Cardinal', 2 (2020).

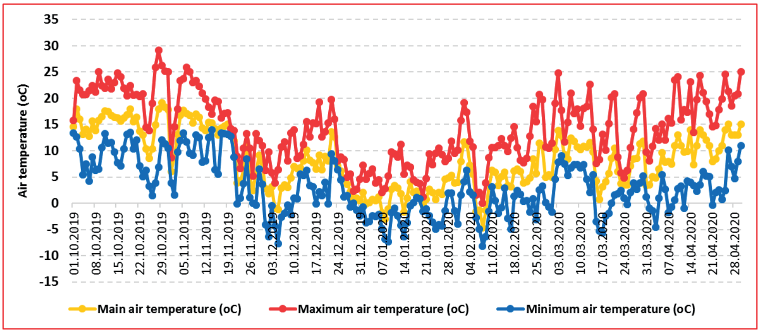
The hardening of the core. This phenophase occurred in the first half of the month of June (between 3th and 10th) in the years 2020, 2021 and 2022.

The harvesting maturity. Calendaristically, each ripening period has large variation limits from one year to another, depending on how the climatic factors determine the type of vegetation in a specific year: early, late or extra late. The harvesting maturity of the fruit had as variation limits the 16th of June and the 15th of August.

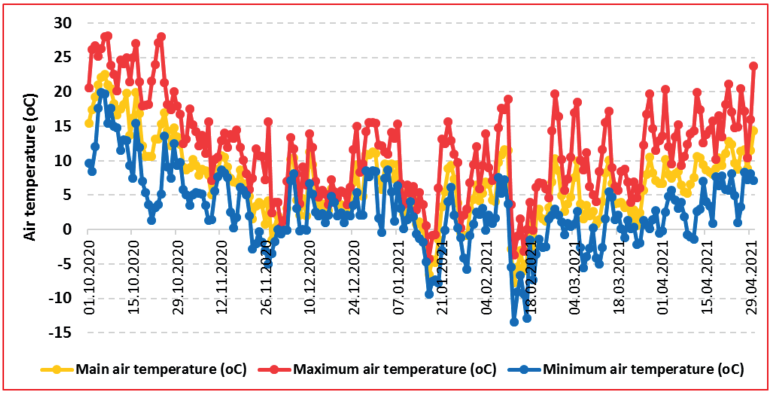
Figure 1a shows that the coldest month was January 2020, with an average minimum temperature of -2.5ºC and an absolute minimum temperature of -7.3ºC. Although the average minimum temperatures for the months of March and April were positive, absolute minimum temperatures of -5.6ºC (17.03.2020) and -4.3ºC (3.04.2020) were recorded, which caused the loss of floriferous buds.

Figure 1b shows that the coldest month was February 2021, with an average minimum temperature of -1.1ºC and an absolute minimum temperature of -13.4ºC. In March, an absolute minimum temperature of -5.5ºC was recorded (7.03.2021), which contributed to the loss of fruit buds.

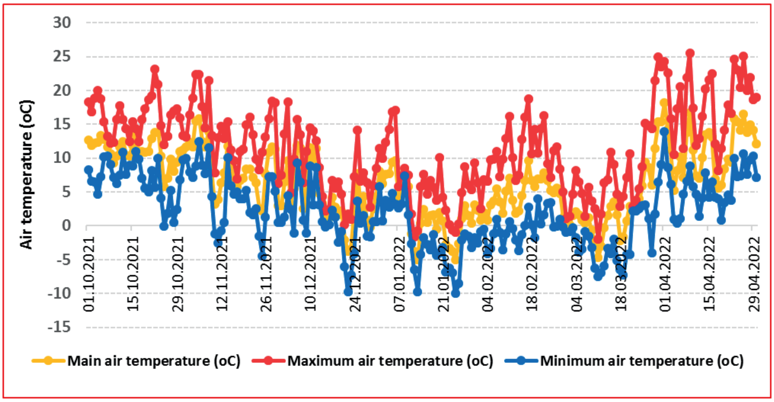
For the period January-April 2022, it can be observed that the coldest month was March, with an average minimum temperature of -2.2ºC and an absolute minimum temperature of -7.5ºC (Figure 1c), which caused the loss of fruit buds.



1a



1b



1c

Figure 1. a, b, c. Air temperature (°C) in October 2019-April 2020 (a), October 2020-April 2021 (b), October 2021- April 2022 (c) at Valu lui Traian, Constanţa

The observations were carried out with the aim of assessing the losses of floriferous buds because of temperature variations during winter and the low temperatures during the day.

Thus, for the ‘Mimi’ cultivar the losses recorded for 2020 were of approximately 40%, 42% for 2021 and 30% for 2022, there being difference from one cultivar to another. The winter frost caused losses for the ‘Catherine Sel. 1’ cultivar of 20% in 2020, 30% in 2021 and 10% in 2022. For the ‘Springcrest’ cultivar, the losses were of 90% in 2020, 80% in 2021 and 60% in 2022. The ‘Raluca’ cultivar recorded losses of 60% in 2020, 24% in 2021 and 24% in 2022. For the ‘Cardinal’ cultivar, the losses were of 80% in 2020, 70% in 2021 and 55% in 2022. The ‘Filip’ cultivar recorded losses of 60% in 2020, 40% in 2021 and 30% in 2022. The 'Cora' cultivar recorded losses of 40% in 2020, 70% in 2021 and 30% in 2022 (Figure 2).

We must bear in mind the fact that the losses caused by the winter frost of 2020, together with those caused by hoarfrosts and late frosts were very severe, taking also into account the surface of the Station's orchards cultivated with this cultivar.

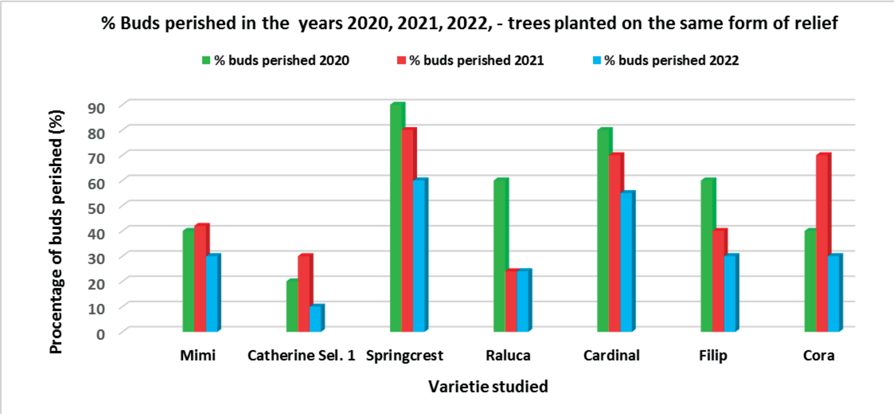


Figure 2. Percentage of peach tree flowering buds perished due to frosts during the winter of 2020, 2021 and 2022 at Valu lui Traian, Constanta

A good resistance to frost during the winter of the three studied years was displayed by the peach cultivar, with the following percentages: 'Catherine Sel.1', 20% and 'Raluca', 24% (Figure 3).

In these conditions, the 'Cardinal', and 'Springcrest' cultivars were more than 63-70% damaged.

At R.S.F.G. Constanta, in the second week of June 2022, more exactly on July 14^{th}, the amount of precipitations was accompanied for 10 minutes by hail, which affected 80% of the fruit production for the Springcrest cultivar (the fruit were just beginning to ripe) and 70% for the 'Cardinal and Cora' cultivar (Figures 4 and 5). The hail bruised the fruit, the shoots and the stems, thus creating a good environment for future infections and diseases. The bruises on the fruit, despite some of them becoming scars, diminished the commercial aspect and the quality of the production.

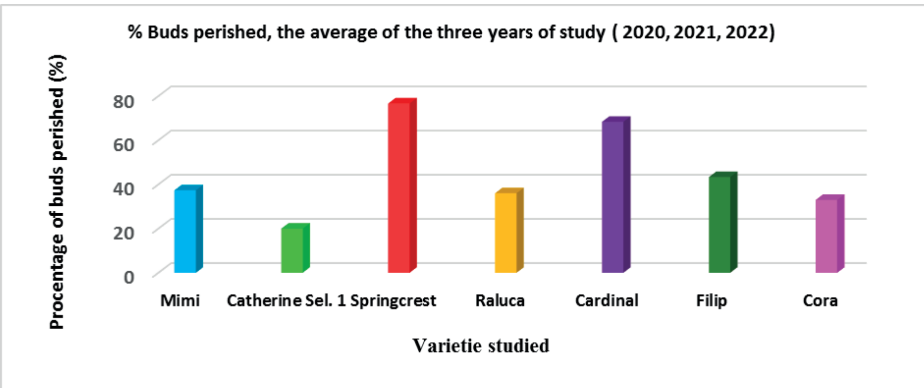


Figure 3. Percentage of peach tree flowering buds perished because of frosts (average over the three years), Valu lui Traian

Figure 4. The ‘Springcrest’ cultivar affected by the hail on July 14th, 2022 (full maturity)

Figure 5. The ‘Cardinal’ cultivar affected by the hail on July 14th, 2022

CONCLUSIONS

The greatest production losses were recorded for the ‘Springcrest’ cultivar in 2020 - 90% in 2021 - 80% and 60% in 2022.

The smallest losses during the three studied years were recorded by the peach tree cultivars ‘Catherine Sel.1’.

The hail from July 14th, 2022, which lasted for only 10 minutes, affected the ‘Springcrest’ cultivar (80%) and the ‘Cardinal and Cora’ cultivar (70%).

These cultivars were affected by the hail that occurred on July 14th, 2022, which facilitated the development of moniliasis especially at cultivars that were in full harvesting maturity.

In order to protect the trees from hail occurrences we recommend that the orchards be equipped with anti-hail nets.

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THE INFLUENCE OF GROWTH REGULATORS ON THE ACHIEVING OF HIGH PRODUCTIONS FROM THE KORDIA CHERRY VARIETY ON THE MAXMA 14 ROOTSTOCK

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Abstract

The object of the researches were cherry trees of the Kordia variety grafted on the MaxMa 14 rootstock. The research was carried out during 2021. To study the effect of the growth regulators Auxiger (1-naphthylacetamide (1-NAD - 1.5 g/l) and 1-naphthylacetic acid (1-NAA - 0.6 g/l)) and Gibbera, SL (gibberellins, 10 g/l mixture of GA4+7) on the degree of fruit setting, development processes, fruit production and economic production efficiency, the following treatment variants were experimented: 1. Control – no treatment; 2. Auxiger, 0.7 l/ha; 3. Gibbera, SL, 0.25 l/ha; 4. Gibbera, SL, 0.5 l/ha. The growth regulator Auxiger was administered only once, during the period of intensive fruit growth, when their diameter reached 12-13 mm (14.05.2021), and Gibbera, SL in three rounds. The first treatment at the end of the flowering phase (26.04.2021), and subsequent two at an interval of 7-10 days, (05.05.2021) and (14.05.2021), respectively. In the reference period it was established that the degree of fruit binding, development processes, higher fruit productions was recorded when treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha.

Key words*: cherry, growth regulator, degree of binding, ripening period, production.*

INTRODUCTION

The cherry is a tree species adapted to the conditions of the temperate zone and has a major importance from an economic point of view. In recent years, world production of cherries has grown rapidly due to the health benefits of their consumption and high profits for producers (Long et al., 2014; Peşteanu et al., 2018).

Cherries are a food, which contains high amounts of nutrients such as vitamin C, polyphenols, carotenoids, potassium and fibre, while being comparatively low in calories. Cherries are also rich in tryptophan, serotonin and melatonin (Cimpoies, 2018).

In determining the importance of the crop, cherry production and fruit size are the main indicators that growers pay attention to (Balan, 2015; Long et al., 2014). Cherry growers tend to maximize plantation productivity, however, there is a negative correlation between yield per tree and fruit quality. In addition, the productivity of the cherry plantation can be quite variable depending on the variety/rootstock association, years and production areas (Budan & Grădinăriu, 2000).

Most cherry varieties produce a high level of ethylene during flowering. Ethylene has a negative influence on flowers, registering their premature aging, which ultimately results in low pollination and binding to flowers (Neamțu & Irimie, 1991).

We record cherry yields below the expected level when there was inadequate pollination by insects in the plantation, low viability and germination of pollen, unsuitable climatic conditions for the uniform growth of the pollen tube, rapid senescence of ovules, lack of overlap of flowering with pollinators, or insufficient number of pollinators. To solve the respective problems, it is necessary to plant 2 pollinating varieties and increase the density of pollinating trees (Budan & Grădinăriu, 2000; Cimpoies, 2018; Long et al. 2014; Long et al., 2020).

In order to extend the period of successful pollination and increase the productivity of the plantation and the quality of the fruits, the use of growth regulators is recommended (Neamțu & Irimie, 1991; Peşteanu & Lozan, 2021; Sabir et al. 2021). Different concentrations of growth regulators administered at optimal times can influence the degree of flower setting, fruit size, fruit shape, fruit firmness and weight, fruit ripening period, including their handling (Zhang & Whiting 2011).

There are multiple studies on the effects of growth regulators in fruit trees, and the results vary depending on the PGR, the time of application, the concentration and even the application technology. Gibberellins also play a vital role in pollen germination, fruit formation and cherry fruit development (Greene, 1988; Zhang & Whiting, 2013).

Preharvest applications of gibberellins to cherries increased fruit size and delayed ripening (Kappel & MacDonald, 2007; Peșteanu et al., 2018; Sabir et al., 2021; Stern et al., 2007) Ovule viability and the degree of fruit set in cherry can be influenced by treatment with GA3, GA4+7 and the mixture of these gibberellic acids. Compared to other GA isomers alone, the combination of GA3 and GA4+7 improves ovule longevity and increases cherry production (Sabir et al., 2021).

The application of GA4+7 slows down the senescence process of the leaves in the year of application and increases the average weight of the leaf blade compared to the control (Neamțu & Irimie, 1991).

Gibbera SL growth regulator is recommended especially for apple, but is now widely used in other fruit species as well. The product contains 10 g/l gibberellic acids GA4 and GA7 and is designed to support plants during flowering and fruiting. Gibbera, SL stimulates the formation of fruits, increases the number of buds, ovaries and fruits, improves the growth and formation processes, accelerates the ripening time, increases the yield, as well as prevents cracking and discoloration of the fruits, improves the appearance and quality of the products (Peșteanu & Lozan, 2021; Пештяну & Кумпанич, А., 2021). The objective of this research was to evaluate the potential effects of different concentrations of GA4+7 on the increase in the number of bound ovaries and fruits in the crown, the intensification of growth processes and the maintenance of the physiological balance in the crown of the tree, the increase of the yield and the quality of production in cherry plantations from the Kordia variety, trees grafted onto MaxMa 14 rootstock.

MATERIALS AND METHODS

The researches were carried out during the 2021 vegetation period in a cherry orchard, planted in the spring of 2015 with annual trees in the form of a rod. Trees of the Kordia cherry variety, grafted on the MaxMa 14 rootstock, served as the object of research. Planting distance 5.0 x 3.0 m. The trees were guided according to the usual spindle crown system. In accordance with the elaborated experience scheme, the following variants were tested (Table 1).

The plots were placed randomly, consisting of 4 repetitions in each variant. Each repetition consisted of 5 model trees. The boundaries between the experimental plots were isolated from the rest of the orchard by a row in which no growth regulator treatments were applied. An untreated tree was left between replicates to prevent accidental spraying of treated trees with other variants and surfaces during treatments.

Table 1. Experimental scheme for determining the effectiveness of the growth regulator Gibbera, SL, for stimulating growth-formation processes, accelerating the ripening time of cherries

Variants	Activ substance	Method and date of application
Control - no treatment	-	-
Auxiger LG - 0,7 l/ha	1-naftilacetamidă (1-NAD – 1,5 g/l) and acid 1-naftilacetic (1-NAA – 0,6 g/l)	By spraying during the period of intensive fruit growth (14.05.2021)
Gibbera, SL - 0,25 /ha	(GA_{4+7}), 10,0 g/l	The first spraying, at the end of the flowering phase (26.04.2021), and two subsequent ones at an interval of 7-10 days, (05.05.2021) and (14.05.2021), respectively
Gibbera, SL - 0,50 l/ha		

The treatment of the trees on the experimental sector was carried out with the portable sprinkler in the morning hours, when there was no wind.

The amount of solution expected when treating a tree with the growth regulators studied to stimulate the growth-formation processes, accelerating the ripening time of cherries was 1.6 litres, resulting from the number of trees per unit area and the amount of water recommended by 1000 l/ha.

The researches were carried out in field and laboratory conditions according to accepted methods of conducting research on fruit crops where the growth regulators were tested.

The leaf surface study was determined according to the method described by V. Balan (2009).

Average and total one-year growth length was determined by measurements on 4 model trees of each variety.

Observations to determine the degree of flowering of the trees were carried out during the white button period and after the fall of the ovules at the beginning of June.

Cherry production was determined at the stage of full fruit ripening by recording the weight. The production from each tree was collected separately and weighed, then the average production for each variety was determined and converted to tons per hectare.

Average fruit weight was determined by weighing 100 fruits and then dividing the result by 100, in four replicates.

The fruits were harvested in two periods, as they ripened. The proportion of fruit harvested in the first and second periods was determined by weighing and counting for the model trees in each replicate.

The economic efficiency of the production of cherries of the Kordia variety was established by the method of calculating the investments made in the cherry plantation in the year 2021.

The obtained results were processed statistically by the dispersion analysis method.

RESULTS AND DISCUSSIONS

Among the main photometric characteristics of fruit plantations, the leaf area and the length of annual growths are of particular importance, since the productive potential of the orchard depends on these indicators.

The obtained experimental data (Table 2) demonstrate that the smallest leaf area within a tree was recorded in the control variant - 10.8 m^2/tree.

Treating the variant with the growth regulator Auxiger LG at a dose of 0.7 l/ha resulted in an insignificant increase in the leaf surface (11.1 m^2/tree), i.e. a 2.8% increase compared to the control variant.

The use of the growth regulator Gibbera, SL in doses of 0.25 l/ha and 0.5 l/ha increased the leaf area compared to the control variant and the variant treated with the growth regulator Auxiger LG in a dose of 0.7 l/ha. In the case of the variant treated with the growth regulator Gibbera, SL, in a dose of 0.25 l/ha, the leaf surface increased by 10.2% compared to the control and by 7.2% compared to the variant where the Auxiger LG product was administered in dose of 0.7 l/ha.

When the variant was treated with the growth regulator Gibbera, SL at a dose of 0.5 l/ha, the leaf area increased to 12.6 m^2/tree, or 13.6% higher compared to the treated variant with the growth regulator Auxiger LG in a dose of 0.7 l/ha and with 5.9%, respectively with the variant treated with the product Gibbera, SL in a dose of 0.25 l/ha.

In the case of treatment with the growth regulator Gibbera, SL in a dose of 0.25 l/ha and 0.5 l/ha, the difference between the studied variants of this index was 0.7 m^2/tree, which was also confirmed by statistical data of mathematical processing.

To obtain stable productions, fruit plantations must form 25-30 thousand m^2/ha of leaf surface. The conducted research demonstrates that in the control version, without treatment, the smallest leaf area was recorded - 7.2 thousand m^2/ha.

The use of the growth regulator Gibbera, SL at a dose of 0.25 and 0.5 l/ha resulted in an increase in leaf area compared to the control variant. In the case of the variant treated with the growth regulator Gibbera, SL, in a dose of 0.25 l/ha, the leaf surface per surface unit increased by 0.7 thousand m^2/ha compared to the control variant and by 0.5 thousand m^2 /ha compared to the variant where the Auxiger LG product was administered in a dose of 0.7 l/ha.

The leaf area registered in the variant treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha increased the index in the study by 1.2 thousand m^2/ha compared to the control variant, and in the case of the variant Auxiger LG in a dose of 0.7 l/ha, with 1.0 thousand m^2/ha.

The development of annual growth is another important indicator that changes under the influence of the studied growth regulators. Stresses during the growing season, such as: drought, high temperatures, insufficient soil moisture, blocking the activity of mineral

elements, aggravate the physiological processes in plants and slow down the development of annual growth. Only treatment with growth regulators can contribute to the improvement of biochemical processes in the plant and a more uniform development of physiological processes.
The study carried out on the average and summed length of one-year increases demonstrates that the growth regulators used in the research influence the studied indicator in a different way (Table 2).
The lowest value of the average length of the annual growth was registered in the control variant, without treatment - 80.0 cm, and the most developed branches in the variants treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha.
The research carried out proves that the average length of the annual growths in the variant treated with the growth regulator Auxiger LG in a dose of 0.7 l/ha was 82.0 cm, which represents an increase of 2.5% compared to the control variant.
In the case of the variant treated with growth regulator Gibbera, SL at a dose of 0.25 l/ha, the average length of annual growth increased by 7.4% compared to the variant Auxiger LG at a dose of 0.7 l/ha and with 10.0% compared to the control variant.

Table 2. Influence of growth regulators on leaf area and annual growth in the Kordia variety cherry plantation

Variants	Leaf surface		Length of annual growths	
	m^2/tree	thousands m^2/ha	cm	m/tree
Control	10.8	7.2	80.0	20.0
Auxiger LG - 0.7 l/ha	11.1	7.4	82.0	20.5
Gibbera, SL - 0.25 l/ha	11.9	7.9	88.0	22.0
Gibbera, SL - 0.50 l/ha	12.6	8.4	94.0	23.5
LDS 0,05%	0,47	0,36	3,9	0,94

The use of the growth regulator Gibbera, SL in a dose of 0.5 l/ha, led to obtaining the highest values (94.0 cm) of the average length of the annual growths, being 6.9% higher compared to the variant treated with the growth regulator Gibbera, SL at a dose of 0.25 l/ha, by 14.7% with the variant Auxiger LG at a dose of 0.7 l/ha and by 17.5% compared to the control variant. These results are also proven by statistical data.
The summed length of one-year growths correlates with the number and average length of one-year branches formed in the crown of the tree, which changes under the influence of the treatments with the studied growth regulators. Lower values of the total length of annual growth during the study period were recorded in the control variant, and higher in the variants treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha. If in the control version the total length of annual growth was 20.0 m/tree, in the version treated with the growth regulator Auxiger LG, in a dose of 0.7 l/ha - 20.5 m/tree, then in the versions where the product Gibbera, SL was administered in a dose of 2.5 and 0.5 l/ha - 22.0 and 23.5 m/tree, respectively. This phenomenon is explained by the fact that the variants treated with the growth regulator based on gibberellic acid improve the physiological processes that take place during the intensive growth of the shoots, namely during the months of May-June.
The results recorded in the version treated with the growth regulator Gibbera, SL in a dose of 0.25 l/ha show that the total length of annual growth decreased compared to the version treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha by 1.5 m/tree, but increased compared to the version where the Auxiger LG product was used in a dose of 0.7 l/ha by 2.0 m/tree, and compared to the control version by 3.5 m/ tree.
The study carried out in the spring of 2021 showed that in the crowns of cherry trees of the Kordia variety, a sufficient number of flower buds were differentiated and no significant deviations were observed on the variants taken in the study, ranging from 7990 pcs/tree in the case the variant in which the treatment with the growth regulator Gibbera, SL in a dose of 0.5 l/ha was foreseen and it increased to 8020 pieces/tree in the variant Gibbera, SL in a dose of 0.25 l/ha (Table 3).
The treatments carried out in accordance with the research program demonstrated that in the variants treated with the growth regulator Gibbera, SL in a dose of 0.25 and 0.5 l/ha, allowed the formation of a different number of fruits in the crown of the trees.
A smaller number of fruits in the crown of cherry trees of the Kordia variety was recorded in the control variant - 1786 pcs/tree and in the

variant treated with the growth regulators Auxiger LG in a dose of 0.7 l/ha - 1800 pcs/tree. The number of fruits in the variants treated with the growth regulator Gibbera, SL, based on gibberellic acid, varied from 1900 to 2023 pcs/tree, i.e. there was an increase of 6.4 - 13.3% compared to the control variant.

Examining separately the effect of the growth regulator on the number of fruits formed in the crown of cherry trees of the Kordia variety, it is observed that approximately identical values as in the control variant were recorded in the variant treated with the growth regulator, Auxiger LG in a dose of 0.7 l/ha - 1800 pcs/tree. This phenomenon is explained by the fact that treatment with this growth regulator is recommended only once during the period of intense fruit growth (fruit diameter of 12-13 mm) and has no positive effect on increasing the degree of binding and the number of fruits from the crown of trees of the Kordia variety.

Table 3. The influence of growth regulators on the number of fruits and the percentage of their binding in the crown of cherry trees of the Kordia variety

Variants	Number of flowers, pcs./tree	Number of fruits, pcs./tree	Binding percentage, %
Control	8009	1786	22.3
Auxiger LG LG - 0,7 l/ha	7995	1800	22.5
Gibbera, SL - 0.25 l/ha	8020	1900	23.7
Gibbera, SL - 0.50 l/ha	7990	2023	25.3
LDS 0.05%	375	84,1	-

The obtained results demonstrate that gibberellic acid GA4+7 has a positive effect on the number of fruits formed in the crown of cherry trees. In the case of using the growth regulator Gibbera, SL in a dose of 0.25 l/ha, the number of fruits formed was 1900 pieces/tree, which represents an increase of 6.4% compared to the control variant and 5.6 % in the case of the Auxiger LG variant in a dose of 0.7 l/ha.

In the case when the trees were treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha, there was a 13.3% increase in the number of fruits tied in the crown of the trees compared to the control variant. Compared to the variant treated with the growth regulator Auxiger LG at a dose of 0.7 l/ha, this indicator increased by 12.3%, and compared to the variant Gibbera, SL at a dose of 0.25 l/ha, with 6.5%.

This increase in the degree of flower binding in the variants treated with the growth regulator based on gibberellic acid GA_{4+7}, Gibbera, SL in a dose of 0.25 and 0.5 l/ha also influenced the obtaining of a greater number of fruits in the crown of trees of the Kordia variety compared to the control variant and the variant treated with the growth regulator Auxiger LG at a dose of 0.7 l/ha.

Lower values of the degree of fruit set was recorded in the control variant (22.3%), which in turn was followed by the variant treated with the growth regulator Auxiger LG in a dose of 0.7 l/ha (22.5%).

The variant treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha recorded the highest values of the index in the study (25.3%), and in the case of the treatment dose of 0.25 l/ha the weight of the fruits. bound decreased (23.7%), i.e. an average value was recorded between the previous variants and the control variant.

The results obtained do not allow to conclude that the treatment with the growth regulator based on gibberellic acid GA_{4+7}, Gibbera, SL in a dose of 0.5 l/ha had a positive impact on the stimulation of fruit shapes, respectively on the binding percentage of them. in the crown of the tree and finally on the number of fruits formed.

It was suggested to study yield and average fruit weight, as there are dogmas in the literature that if trees are treated with a growth regulator whose active ingredient is gibberellic acid (GA_{4+7}), fruit development is blocked, production in within a tree and per unit area is reduced and can negatively influence the differentiation of fruit buds for the next year, i.e. a weaker flowering is expected. Other studies show conflicting results compared to the hypothesis mentioned above.

The studies carried out demonstrated that the studied growth regulators, Auxiger LG and Gibbera, SL positively influence the average fruit weight and production in general.

The average fruit weight in the studied variants underwent insignificant changes and was correlated with the number of fruits obtained within the tree and the growth regulator used in the treatment.

The investigations carried out highlight the fact that higher values of the average weight of the

fruits were recorded in the Auxiger LG version in a dose of 0.7 l/ha - 9.4 g. In the control version, the average weight of the fruits was 8 .9 g. Productivity is an indicator that shows how all agronomic measures have been applied in the Kordia cherry orchard.
The previously presented results showed that the highest number of fruits was obtained in the variants in which treatments were carried out with the growth regulator based on gibberellic acid GA_{4+7}, Gibbera, SL in a dose of 0.25 and 0.5 l/ha, 1900 and 2023 pcs/tree, respectively, compared to the other variants (tab. 3). Consequently, the average fruit weight decreased insignificantly in these variants, where it constituted 8.9 and 8.7 g, respectively (Table 4).

Table 4. The influence of the growth regulator on the number of fruits, the average weight and the production of cherries of the Kordia variety

Variants	Number of fruits, pcs/tree	Average weight, g	Fruit production		In % compared to control
			kg/ tree	Fruit production, t/ha	
Control	1786	8.9	15.9	10.6	100.0
Auxiger LG - 0.7 l/ha	1800	9.4	17.3	11.5	108.5
Gibbera, SL - 0.25 l/ha	1900	8.9	16.9	11.3	106.6
Gibbera, SL - 0.50 l/ha	2023	8.7	17.6	11.7	110.4
LDS 0.05%	87	0.22	0.73	0.49	-

In the case of using the growth regulator Gibbera, SL in a dose of 0.25 l/ha, the average fruit weight was at the same level as in the control variant, but the number of fruits was much higher, which proves that the products based on of gibberellic acid have an influence on the average weight of the fruits and the yield of the trees.
Because, the control variant, recorded a reduced number of fruits within the crown of the trees, respectively, in this variant, lower productions were obtained per tree (15.9 kg) as well as per surface unit (10.6 t).
The investigations carried out highlight the fact that the treatment carried out with the growth regulator Gibbera, SL in a dose of 0.25 l/ha, recorded lower values of fruit production compared to variants 2 and 4, but higher compared to the control variant, constituting 16.9 kg/tree and 11.3 t/ha, respectively.
In the variant treated with the growth regulator Auxiger LG at a dose of 0.7 l/ha, due to the lower number of fruits per tree and the significant increase in the average fruit weight, it had a positive impact on the production obtained per tree (17.3 kg) and productivity per surface unit (11.5 t/ha), a fact also confirmed by statistical data. The values obtained in the respective variant were approximately equal to those recorded in the variant treated with the growth regulator Gibbera, SL in a dose of 0.25 l/ha.
The investigations carried out highlight the fact that the variant treated with the growth regulator Gibbera, SL in a dose of 0.5 l/ha registered a slight decrease in the average weight of the fruits compared to the other variants, and due to the greater number of fruits recorded a significant increase in production per tree (17.6 kg) and per surface unit (11.7 t/ha).
In the case of the study of the effect of the treatment dose on the recorded yield, the difference between the variants treated with the growth regulators Auxiger LG in the dose of 0.7 l/ha and Gibbera, SL in the dose of 0.5 l/ha is not recorded, which is also confirmed by statistical data.
That is, the difference between the fruit production obtained between the control variant and the variant treated with the product Auxiger LG in a dose of 0.7 l/ha was 8.5%, in the variant Gibbera, SL in a dose of 0.25 l/ha - 6.6%, and in the Gibbera version, SL in a dose of 0.5 l/ha, the corresponding value reached 10.4%.
The optimal moment for harvesting cherries is considered ripe for consumption, because after the fruit is easily separated from the fruit formation, no physiological process takes place in it to improve its quality.
In 2021, the flowering of the cherries was triggered over a longer period of time and therefore the ripening was more staggered. Therefore, the cherries were harvested in two stages, when they had the characteristic color of the variety and a better flavor.
The investigations carried out highlight the fact that the treatment of the experimental variants with the growth regulators Auxiger LG in a dose of 0.7 l/ha and Gibbera, EW in a dose of 0.25 and 0.5 l/ha influenced the coloring of the fruits. The proportion of fruits harvested in the first and second pass of the picker is considered to be the

most important indicator for that species. The obtained results show that in the control version, 67.5% of the fruits were harvested in the first harvest period (07.05.2021), and the rest, 32.5% in the next installment (07.08.2021) (Table 5).

Table 5. The influence of the growth regulator on the weight of fruits of the Kordia variety collected when reaching the optimal harvest period, %

Variants	Harvesting period	
	05.07.2021	08.07.2021
Control	67.5	32.5
Auxiger LG - 0.7 l/ha	89.5	10.5
Gibbera, SL - 0.25 l/ha	87.4	12.6
Gibbera, SL - 0.50 l/ha	92.4	7.6

Treating the trees with the growth regulator from the Auxiger LG variant in a dose of 0.7 l/ha increased the weight of the fruits harvested at the first pass to 89.5%, an increase of 22.0% compared to the control variant. At the second collection, the share of harvested fruit was 10.5%.

Variants treated with the growth regulator Gibbera, SL, at a dose of 0.25 and 0.5 l/ha, also had a positive impact on the weight of fruits harvested at the first harvest.

If the trees were treated with the growth regulator Gibbera, SL in a dose of 0.25 l/ha, the proportion of fruits harvested at the first pass was slightly lower (2.1%) than in the variant treated with the growth regulator growth Auxiger LG in a dose of 0.7 l/ha and which was 87.4%.

The highest proportion of cherry fruits harvested at the first harvest was recorded in the version treated with the product Gibbera, SL in a dose of 0.5 l/ha, where this indicator constituted 92.4% and 7.6%, respectively, of fruits from the crown of trees.

The economic efficiency of cherry production depends on some strict economic rules that allow to minimize production costs.

The investigations carried out highlight the fact that the growth regulators Auxiger LG and Gibbera, SL had a positive influence on the weight of the bound fruits and the productivity of the plantation, which allowed to obtain a higher sales income compared to the control variant.

In the case of the control variant, the income from sales was 307.4 thousand lei/ha, when treated with the growth regulators Auxiger LG and Gibbera, SL, this figure recorded values of 339.0 - 351.0 thousand lei/ha. Higher values of the income from sales were registered in the Gibbera, SL version in the dose of 0.5 l/ha - 351.0 thousand lei/ha. Next, in decreasing order are the variants treated with the growth regulators Auxiger LG in a dose of 0.7 l/ha - 342.0 thousand lei/ha and Gibbera, SL in a dose of 0.25 l/ha - 339.0 thousand lei/ha (Table 6).

Table 6. Economic production efficiency of cherry fruits from the Kordia variety in the case of treatment with growth regulators

Variants	Sales income, thousands of lei/ha	Cost of production, thousands of lei/ha	Profit, thousands of lei/ha	The profitability, %
Control	307.4	100.4	207.0	206.2
Auxiger LG - 0.7 l/ha	342.0	107.0	235.0	219.6
Gibbera, SL - 0.25 l/ha	339.0	105.5	233.5	221.3
Gibbera, SL - 0.50 l/ha	351.0	107.2	243.7	227.1

The investigations carried out highlight the fact that for the treatment with the growth regulator Gibbera, SL in the dose of 0.25 l/ha - 0.3 thousand lei/ha was invested in the procurement of the product, for the variant Gibbera, SL in the dose of 0.5 l/ha - 0.6 thousand lei/ha, and when treated with the growth regulator Auxiger LG in the dose of 0.7 l/ha - 1.4 thousand lei/ha.

The lowest production cost was recorded in the control version - 100.4 thousand lei/ha. In the version treated with the growth regulators Gibbera, SL in a dose of 0.25 l/ha, the studied indicator was 105.5 thousand lei/ha. The treatment with the growth regulators Auxiger LG in a dose of 0.7 l/ha and Gibbera, SL in a dose of 0.5 l/ha had a significant impact on the production cost (107.0-107.3 thousand lei/ha), as the additional investment to purchase the product and collect the additional fruit took its toll compared to previous variants.

The profit of the product is directly related to its sales revenue and production cost. Lower profit values were recorded in the control version - 207.0 thousand lei/ha, and higher in the version treated with the growth regulators Gibbera, SL in a dose of 0.5 l/ha - 243.7 thousand lei/ha. The variants treated with the growth regulators Gibbera, SL in a dose of 0.25 l/ha and Auxiger LG in a dose of 0.7 l/ha, recorded a profit of 233.5 and 235.0 thousand lei/ha, respectively.

The effectiveness of the treatment with the growth regulator Gibbera, SL in the dose of 0.5 l/ha is also confirmed by the level of profitability. While the level of profitability of the control variant was 206.2%, in the case of the use of growth regulators the indicator in the study varied from 219.6 to 230.0%, but maximum values were recorded in the variant treated with the product Gibbera, SL in a dose of 0.5 l/ha - 227.1%.

CONCLUSIONS

Based on the experimental results, the growth regulator Gibbera, SL can be included in the technological scheme of cherry plantation cultivation to increase the degree of fruit binding, intensify the processes of growth and formation, and accelerate the ripening of cherry fruits in the dose of 0.5 l/ha, applied 3 times by foliar treatment. The first treatment to be carried out at the end of the flowering period, and the next 2 at an interval of 7-10 days after the previous one.

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CONTENT OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN CHOKEBERRIES JUICE (*ARONIA MELANOCARPA*)

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Abstract

Aronia fruits (Aronia melanocarpa) are very rarely used in the Romanian diet, although they have the highest antioxidant properties of all fruits. Therefore, the objectives of this study were to evaluate the bioactive compounds and antioxidant activity of fresh aronia juice and dried fruit residues. The antioxidant activity of the samples was determined by DPPH assay. Total antioxidant capacity was evaluated by the phosphomolybdate method. The content of polyphenols, flavonoids and anthocyanins in the aronia samples was also investigated. The results showed that fresh aronia juice contains the highest levels of phenols, flavonoids and antioxidant activity. A high level of anthocyanins was found especially in the dried fruit residues. These results demonstrate the potential of Aronia melanocarpa as a healthy and nutritionally rich dietary food with many functionalities and benefits.

Key words*: Aronia melanocarpa, phenols, flavonoids, anthocyanins, antioxidant activity.*

INTRODUCTION

Aronia (*Aronia melanocarpa*), commonly called black chokeberry, belongs to the *Rosaceae* family and is a shrub native to North America whose fruits are highly sought after and valued for their wonderful therapeutic properties. Aronia was introduced in Europe about a century ago (Kulling & Rawel, 2008; Sainova et al., 2012; Kim et al., 2013). Aronia culture, relatively new in our country, is conquering more and more farmers, especially those with not very large plots of land. Thus, they may have the opportunity to come to the market with some fruits quoted at a better price and recognized for their extraordinary therapeutic properties. The components of the plant contain several useful bioactive compounds such as polyphenols, flavanols, and anthocyanins (Kulling & Rawel, 2008; Sharif et al., 2013; Malinowska et al., 2013) with a positive effect on the human health. Previous studies on aronia have reported that the leaves of several Aronia species are used in traditional medicine because of their anti-inflammatory, antiviral, antimicrobial, and antiproliferative activities against cancer cells (Ljubuncic et al., 2005; Martini et al., 2009). Therefore, aronia leaves might contain bioactive compounds and have biological effects resulting from the polyphenols, flavonoids, and chlorophylls that they contain. Other studies focused on the juice of the aronia fruit (Sainova et al., 2012; Kim et al., 2013; Sharif et al., 2013), aronia leaves (Nhuan Do Thi & Eun-Sun Hwang, 2014) and on Aronia wastes obtained after juice extraction because these products contain many phenolic compounds, including anthocyanins (D'Alessandro et al., 2013). Anthocyanins are phenolic compounds which are water-soluble pigments. They are responsible for imparting a variety of colors to the plants like orange, red, pink, blue and purple. It is well known that anthocyanins possess antioxidant activity and have different other pharmacological properties. Anthocyanins can be used industrially as natural colors and can be used for a wide variety of foods, cosmetics, and drugs. As a natural product, anthocyanins are good for health due to their antioxidant properties and may have a role in immunity by boosting our immune system (Wegdan et al., 2020). Data on the antioxidant activity and phenolic content of chokeberry have been reported in several studies (Oszmianski & Wojdylo, 2005; Jakobek et al., 2007; Denev et al., 2012).

In this context, the aim of this study was to evaluate the content of total phenolics, flavonoids, and anthocyanins as well as antioxidant properties of chokeberry fresh juice and dried residue material.

MATERIALS AND METHODS

Plant material. The fresh chokeberry fruits of the 'Melrom' variety were purchased from the Research and Development Station for Fruit Tree Growing Băneasa, Bucharest (Figure 1).

Figure 1. Fresh fruits of *Aronia melanocarpa* "Melrom" variety

Samples preparation. 60 g of fruits were weighed and blended to obtain juice. The aronia residue was dried at 100°C until 1 g of dry substance was obtained. Then 1 ml of juice and 1 g of dry material were each mixed with 10 ml of methanol acidified with 2% HCl. The obtained fresh juice and the acidified methanol extracts were used for the determination of the total phenolic content (TPC), total flavonoids (TFC), total anthocyanins (TA), as well as for the determination of the antioxidant activity.

Determination of total phenolics. For determination of TPC, a method with Folin-Ciocalteu reagent (Sigma-Aldrich) (Singleton, 1999) was used. An aliquot (20 µL) of diluted chokeberry sample or standard solutions of gallic acid (25-500 mg/L) was mixed with 1580 µL of distilled water and 100 µL of Folin-Ciocalteu reagent. A volume of 300 µL of sodium carbonate solution was added to the mixture. After incubation at room temperature for 2 h, the resulting absorbance was measured by the spectrophotometer (Eppendorf UV-Vis) at the wavelength of 765 nm against the blank sample, which was used as reference. The results were calculated according to the calibration curve for gallic acid as follows: y = 0,0012x + 0,0153; R = 0,9994 (Figure 2), where: y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/L; R2=0.9994. Total phenolics were expressed as mg of gallic acid equivalents (GAE) per l of chokeberry juices and as mg of GAE per g of dry matter (dm).

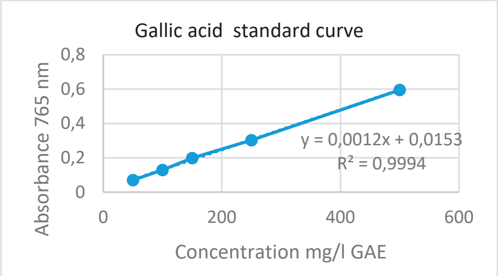

Figure 2. Gallic acid standard curve and the regression equation

Determination of total flavonoids content. The total content of flavonoids in the samples was determined by the colorimetric method with aluminum chloride (Chang et al., 2002). Quercetin was used as a standard agent, and the total flavonoid content was expressed in µg quercetin equivalent/ml. The reaction mixture consisted of: 1 ml sample/standard, 3 ml methanol, 200 µl $AlCl_3$, 200 µl 1 M potassium acetate and 5.6 ml distilled water. The absorbance of this reaction mixture was recorded at 420 nm using a UV spectrophotometer (Eppendorf UV-VIS). The concentration of flavonoids (mg quercetin equivalent/ml) in the samples was determined based on the standard calibration curve (y = 0.009x + 0.0538; R^2 = 0.9913) obtained for different concentrations of quercetin (25, 50, 100, 150 and 200 mg/ml) (Figure 3) and as mg of QE per g of dry matter (dm).

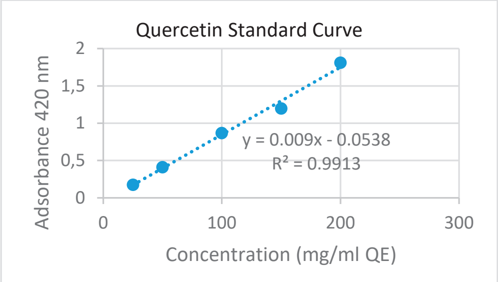

Figure 3. Quercetin standard curve and the regression equation

Determination of total anthocyanins content. Total anthocyanins content (TA) was carried out using the pH differential spectrophotometric method (Giusti & Wrolstad, 2000). Each sample was diluted 10 times to a final volume of 2 ml in two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5). The diluted portions were filtered using PTFE membrane filters (0.2 mm). After filtration the absorbance of each diluted solution was measured at 520 nm against distilled water as blank and corrected for haze by measuring the absorbance at 700 nm. TA in juice was calculated according to the following formula and expressed as mg **cyanidin-3-glucoside** equivalents (CGE) per ml of chokeberry samples and as mg of CGE per 100 g of dm:

$$\frac{A\ x\ Mw\ x\ DF\ x\ 10^3}{\mathcal{E}\ x\ l}$$

Where:

A (Abs) = ($A_{520\ nm} - A_{700\ nm}$)pH 1.0 – ($A_{520\ nm} - A_{700\ nm}$) pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF (dilution factor) = 10; l (pathlength) in cm; ε (molar extinction coefficient) = 26,900 L × mol^{-1} × cm^{-1} for cyanidin-3-glucoside; and 10^3 = conversion factor for g to mg.

Antioxidant activity

DPPH method. The free radical scavenging activity of the samples taken in the study was determined with 1, 1-diphenyl-2-picrylhydrazyl (DPPH), described by Braca et al. (2001). A volume of 200 µl of each sample of different concentrations (10-100 µg/ml) with 2 ml of 0.004% methanol solution of DPPH (0.1 mM). After 30 minutes of incubation in the dark at room temperature, the color change from dark purple to light yellow was determined at 517 nm against 1 ml methanol (blank) using a UV spectrophotometer (Eppendorf UV-VIS). Different concentrations of ascorbic acid (10-200 µg/ml) were used as a standard agent. The scavenging ability (%) was calculated as follows:

$$\%\ Inhibition = \frac{Standard\ absorbance - Crude\ extract\ absorbance}{Standard\ absorbance}$$

Ascorbic acid was used as positive standard. The antioxidant capacity of the samples was expressed as inhibitory concentration, IC_{50} µg/ml. The lower IC_{50} value indicates the greater overall effectiveness of the antioxidant. ***Total antioxidant capacity***. The total antioxidant capacity of the samples was evaluated by phosphomolybdate method (Prieto et al., 1999) using ascorbic acid as a standard (Garrat, 1964). The reaction mixture consisted of 0.3 mL extract combined with 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. After the samples cooled to room temperature, the absorbance of the solution was measured at 695 nm against the blank using a spectrophotometer. Methanol (0.3 ml) was used as control. The results were expressed in ascorbic acid equivalent in µg/ml extract based on the standard calibration curve (y = 0.0067x + 0.0029; R = 0.9982) obtained for different concentrations of ascorbic acid (10-200 µg/ml) (Figure 4). The higher absorbance value indicated higher antioxidant activity.

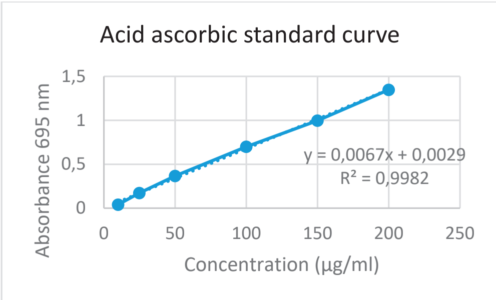

Figure 4. Ascorbic acid standard curve
and regression equation

Statistical Analysis. Results were expressed as standard error of the mean (SEM) for triplicate measurements. The graphics were plotted by using Microsoft Office Excel 2010.

RESULTS AND DISCUSSIONS

Total phenolics, flavonoids and anthocyanins

Total phenolic content values were higher in crude juice (P1) (2.69 ± 0.009 mg GAE/l) than in acidified methanolic juice (P2) (0.182 ± 0.002 mg GAE/l) and dried berries residue (P3) (1.02 ± 0.024 mg GAE/g dry matter). Lower or higher values reported in the literature might have resulted from different extraction methods used for analysis, differences in analytical procedures applied, different processing technologies and storage conditions, or differences in chokeberry cultivars (Denev et al., 2012). Researchers report processing influences the phenolic content of final products reaching consumers (Kobus et al, 2019), it was found that *Aronia melanocarpa* products contain high amounts of polyphenols (Tolić et al., 2015). Tolić et al. (2017) showed how weather conditions, such as temperature and insolation, influenced phenolic content in the juice. It was found that warm and dry climate conditions have a positive impact on the increasing value of total phenolics.

Flavonoid content was predominant, and their amounts varied from 295.92 mg of QE/ml in P1 sample to 57.92 mg of QE/ ml in P2 sample. Average of total flavonoid content in dried berries residue (P3) was 158.8 mg QE/g dry matter. These results suggest that flavonoids were the most abundant in chokeberry fresh juice and in dried berries. In this case, our results are in concordance with other research which showed that the content of total flavonoids was higher in fresh juices and in dried chokeberries (Kapci et al., 2013).

The total flavonoid content was predominant, and their amounts varied from 295.92±6.801 mg of QE / ml in P1 sample to 57.92±0.163 mg of QE/ ml in P2 sample. Average of total flavonoid content in dried berries residue (P3) was 158.8 mg QE/g dry matter. These results suggest that flavonoids were the most abundant in chokeberry fresh juice and in dried berries. In this case, our results are in concordance with other research which showed that the content of total flavonoids was higher in fresh juices and in dried chokeberries (Kapci et al., 2013).

Anthocyanins content

Chokeberries contain relatively higher amounts of ***anthocyanins*** compared to other berries fruits, grape and cherry, which are known as rich sources of anthocyanins (Kulling and Rawel, 2008; Denev et al, 2012). Our results sowed lower amounts of anthocyanins in juice mixed with methanol/2 % HCl (20.15 ± 0.619 mg CGE /ml) and in fresh juice (14.2 ± 1.338 mg CGE /ml) and a higher concentration of anthocyanins accumulated in fruit residue (155.9±5.891 mg CGE/100 g dry matter). Cyanidin-3-galactoside and cyanidin-3-arabinoside are predominant in the berries with a cumulative content >90 % (Denev et al., 2012). As a natural product, anthocyanins are good for health due to their antioxidant properties and may have a role in immunity by boosting our immune system (Wegdan Ali Shehata et al. 2020). The content of total phenolics (TPC), total flavonoids (TFC) and total anthocyanins (TA) in aronia raw juice and in acidified methanolic extracts of juice and dried material are presented in Table 1 and in Table 2.

Table 1. Total content of phenol (TPC), flavonoids (TFC) and anthocyanins (TA) in aronia fresh juice

Sample	TPC mg GAE/l	TFC mg QE/ml	TA mg CGE/ml
P1	2.69±0.009	295.92±6.801	14.2±1.338
P2	0.182±0.002	57.92±0.163	20.15±0.619

The values are presented as mean ± SEM for triplicate measurements.
P1 - chokeberry fresh juice, P2 - chokeberry juice mixed with methanol/2% HCl.

Table 2. Total content of phenol (TPC), flavonoids (TFC) and anthocyanins (TA) in aronia residue

Sample	TPC mg GAE/g dm	TFC mg QE/g dm	TA* mg CGE/100 g dm
P3	1.02±0.024	158.8±0.136	155.9±5.891

The values are presented as mean ± SEM for triplicate measurements.
P3 - Berries residue with methanol/2% HCl. Contents of TPC and TFC in dried berries residue methanol/2% HCl samples are expressed as mg per g of dry matter (dm) and *TA are expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g of dry matter (dm).

Antioxidant activity

To evaluate the antioxidant activity of *Aronia melanocarpa*, the most commonly used assays include the inhibition of DPPH radicals (2,2-dipheny-l-1-picryl-hydrazyl) (Oszmiański & Wojdylo, 2005). In our experiments, DPPH scavenging ability assay and total antioxidant capacity (TAC) assay were used to evaluate the antioxidant activity of each sample. The results are show in Table 3.

Table 3. Antioxidant Activity by DPPH and TAC assays

Sample	DPPH $IC_{50\%}$ (μg /ml)	TAC (μg/ml)
P1	14.42±0.226	1442.1±1.825
P2	54.21±0.032	61.97±1.770
P3	25.14±0.223	197.8±6.93
AA	12.27±0.294	

Data are mean ± SEM for triplicate measurements.
P1 - chokeberry fresh juice; P2 - chokeberry juice mixed with methanol/2% HCl; P3 - chokeberry dried material with methanol/2% HCl; AA - Ascorbic acid.

In present study, ascorbic acid as a well-known potent antioxidant, was used as positive control for DPPH scavenging activity. Concentration of samples and ascorbic acid (AA) resulting in 50% inhibition on DPPH ($IC_{50\%}$ value) were calculated. The lower IC_{50} value indicates a higher antioxidant activity. Chokeberry raw juice (P1) showed highest ability in DPPH scavenging activity (14.42 ± 0.226 µg/ml) followed by methanolic chokeberry dried residue (P3) (25.14±0.223 µg/ml) compared to methanolic juice sample (P2) which measured by the lowest IC_{50} value (54.21 ± 0.032 µg/ml), but it has lower antioxidant capacity compared to ascorbic acid (12.27 ± 0.294 µg/ml) (Figure 5).

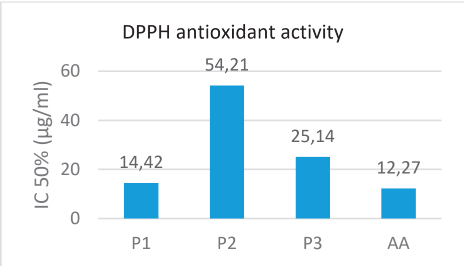

Figure 5. DPPH antioxidant activity in aronia samples

(P1 - chokeberry fresh juice; P2 - chokeberry juice mixed with methanol/2% HCl; P3 - chokeberry dried material with methanol/2% HCl; AA - Ascorbic acid)

The phenolic content in the raw juice and residue samples may contribute to the antioxidative action by hydrogen donating ability. The high content of polyphenols is responsible for the strong antioxidant properties of chokeberries and their products (Tolić et al., 2017).
The phosphomolybdate method has been used routinely to evaluate the total antioxidant capacity of plant extracts (Prieto et al., 1999; Prasad et al, 2009). The total antioxidant capacity (TAC) of different chokeberry samples is shown also in Table 2 and in Figure 6. The higher absorbance value indicated higher antioxidant activity (Prasad et al., 2009). Examined samples showed that the highest total antioxidant activity is rich by chokeberry raw juice (P1) (1442.1 ± 1.825 µg/ml) and methanolic dried material (P3) (197.8±6.93 µg/ml), and the lowest total antioxidant activity was in methanolic/2% HCl chokeberry juice (P2) (61.97 ± 1.770 µg/ml) (Figure 6).
These results are in accordance with other reports in the literature, which showed positive strong correlation between antioxidant activities and total polyphenol contents (Zhao et al, 2008).

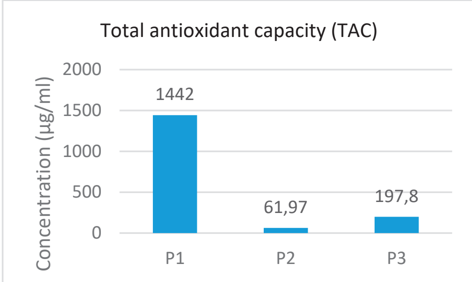

Figure 6. Total antioxidant capacity (TAC) of different chokeberry samples
(P1 - chokeberry fresh juice, P2 - chokeberry juice mixed with methanol/2% HCl; P3 - chokeberry dried material with methanol/2% HCl)

CONCLUSIONS

In this investigation, high contents of phenolic and flavonoid compounds and high values of antioxidant properties were observed in the fresh juice of *Aronia melanocarpa*. The presence of anthocyanins was observed in high amounts, especially in the residual material. The results show that the fresh fruits have significant antioxidant properties. Due to their high content of natural antioxidants, their consumption could bring health benefits. The present study contributes to the existing knowledge by providing new data.

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PHENOLOGICAL CALENDAR OF PLUM CULTIVARS, DEPENDING ON THE CHANGES OF CLIMATIC CONDITIONS FOR THE TROYAN REGION, BULGARIA

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Abstract

The purpose of this paper was to analyze the influence of climatic factors and their impact on the phenology of some plum varieties grown in RIMSA Troyan during the period 2019-2021. The phenophases of the beginning of flowering, the end of flowering and the period of fruit ripening were observed and analyzed. Regarding the flowering phenophase, it was found that earliest blossoming of plum cultivars occurred at the beginning of April in 2019. The blossoming period for 'Kyustendilska' both began and ended at the latest time. Every year, within 10-11 days, there was a complete overlap of the blossoming of all the studied cultivars.
Regarding the fruit ripening, higher temperatures combined with low amounts of precipitation, cause an earlier onset of the ripening phase.

Key words*: plum, cultivars, phenology, climate change.*

INTRODUCTION

Agro-climatic adaptability and ecological plasticity of plum cultivars are key elements for research in agriculture and the information is essential for the creation of sustainable and competitive plantations. Jakab-Ilyefalvi et al. (2021) believe that data on the occurrence and duration of phenophases are bioindicators of climate change events and are key elements in fruit crop technology. The large variation in spring temperatures mainly affects early cultivars, which affects the technology, market, prices and investment costs of farmers (Jakab-Ilyefalvi et al., 2021). Temperature factors cannot be controlled directly, therefore suitable cultivars are selected for the establishment of orchards, according to the period of blossoming. Modeling detailed phenology is the key to the correct selection of optimal cultivars in a specific geographic area (Ganji Moghaddam et al., 2011).
Tree fruit species synchronize their physiology, including blossoming and annual development patterns, according to environmental conditions, as temperature is a major factor. Stone fruit species are the earliest to enter the blossoming process. Fadón et al. (2021) reported that the earliest blossoming dates were associated with a large amount of heat following a prolonged cold spell. These findings may support the development of new modeling frameworks that can help predict the impacts of climate change on tree dormancy.
Potential yields of fruit species are also strongly influenced by climate conditions that regulate dormancy and subsequent blossoming. Yield fulfillment at the end of the vegetation season depends on several additional factors, including environmental conditions and cultivation technologies. Information on how the reduction of cold (i.e., global warming) affects crop yield is crucial for farmer decision-makers, especially in warm fruit growing regions (Whitney, 2021).
The date of mass swelling of flower buds occurs after an increase in the average day and night air temperatures above 5ºC and is taken as a sign of the termination of the "forced dormancy" in fruit species. An increase in the average daily temperature above 10.5ºC leads to a sharp increase in the temperature sum. Broad limits for the beginning and end of fruit bud break were found by Kazandzhiev &

Malasheva (2016). Cool and rainy weather prolongs the blossoming phase, and dry and warm weather shortens it. Very rapid overblooming is not favorable, because most of the flowers are not pollinated by insects.

Milatovic et al. (2019) for the conditions of Belgrade, The average flowering time of the tested cultivars was in the first half of April, and the average duration of flowering varied from 7.4 to 10.4 days. Glišić et al. (2016) investigate phenological, pomological and productive properties of three new plum cultivars (‘Zlatka’, ‘Mildora’, ‘Krina’) and one standard (‘Čačanska Rodna’) developed at Fruit Research Institute, Čačak. Maghlakelidze et al. 2017 defines the cultivars a collection plot of the Scientific-Research Center of Agriculture, in village Jighaura of Georgia, according to flowering time: Early (‘Amers’, ‘Bluefree’), Middle (‘Stanley’, ‘President’, ‘Empress’) and late (‘Chanchuri’, ‘Tophit’) cultivars during flowering. there were 12- daily difference between early and late cultivars during flowering.

Dimitrova & Sotirov (2020) found a strong correlation dependence between the temperature sums above 5 and 10°C and the number of days for blossoming and fruit ripening.

The objective of the present study was to determine the phenological response to climate change and the behavior of some plum cultivars in the Central Balkan region for the period 2019-2021.

MATERIALS AND METHODS

Materials

The ‘Rutgerstetter’, ‘Top First’, ‘Katinka’, ‘Tegera’, ‘Hanita’, ‘Strinava’, ‘Gabrovska’, ‘Mirabelle de Nancy’, ‘Stanley’, ‘Kystendilska’, ‘Jojo’, ‘Elena’ plum cultivars have been studied at the RIMSA-Troyan (42°53′N 24°43′E). The trees are grown on light gray forest soil, under nonirrigated conditions, with tillage in the row-spacings.

The investigated plum cultivars find suitable conditions for good growth and development in the Troyan region. Crowns typical for the respective cultivar are formed, with sizes corresponding to the age.

Methods

Observations were made throughout the year for each phenophase. The phenophases - beginning of blossoming, end of blossoming and fruit ripening periods - were established and the number of days was determined according to the generally accepted methodology for studying plant resources in fruit plants (Nedev et al., 1979).

Based on data from the Meteorological Station on the territory of RIMSA, the meteorological factors were analyzed, such as average monthly temperatures and total amounts of precipitation for 2019-2020 compared with a 20-year base period (Figure 1); temperatures, precipitation and atmospheric humidity by ten days in March and April (2019-2020) (Table 1).

RESULTS AND DISCUSSIONS

Climate conditions for the Troyan region are characterized by warm and dry summers and not very cold winters. For the study period, warmer winter months (January and February) were recorded in 2020 (with 1.5-2°C) and 2021 (with 1.5°C) compared to the average for a 20-year base period (Figure 1). In 2021, March and April were very cold, which is a prerequisite for delaying the onset of vegetation.

For the summer months of 2021 (May and June), a complete coincidence of temperatures compared to the base period was reported, while July, August and September were each 2 degrees warmer and without significant amounts of precipitation (only 12 mm), i.e. this period is characterized as drought.

It was found that after mid-summer, during fruit ripening, autumn rainfall was scarce (from 16 to 30mm), except for October 2020 (114 mm). The temperatures in August, September, October in all three years were higher than the basic average, which is a sign of a tendentious global climate warming and changes that will seriously affect the factors on which the favourable cultivation of plums in our area depends.

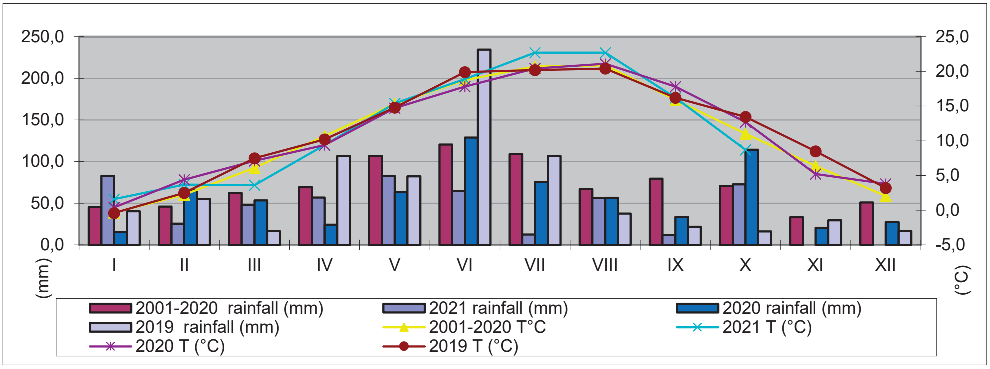

Figure 1. Average monthly temperatures and total precipitation (2019-2021) and 20-year base period (2001-2020)

During the period of the beginning of blossoming, the changes in the factors, such as temperature, humidity and the amount of precipitation for 2019-2021 were different as the difference in precipitation during the first 2 ten days of April 2019 (50.4 mm) and 2020 (10.6-9.2 mm) was distinct. In 2021, very low temperatures (from 3.3 to 6.3°C) were registered in March and the beginning of April, which is the reason that blossoming began in the third ten days of April, when they reached 11.3°C. In 2020, temperatures above 10°C were recorded in the second ten-day period, and then the blossoming begins, because at the beginning of the vegetation season, they are from 5.3 to 5.9°C, and in 2019, even at the beginning of April, the average temperature for the first ten-day period was 9.8°C, which is a prerequisite for early blossoming (Table 1).

Table 1. Meteorological factors in March and April in ten-day period (2019-2021)

	March			April		
2019	1-10	10-20	21-31	1-10	10-20	21-30
Relative humidity (%)	61.0	69.0	62.0	68.0	79.0	70.0
Rainfall (mm)	1.6	14.3	0.6	50.4	45.1	11.4
Temperature (°C)	8.0	7.9	6.8	**9.8**	8.1	12.0
2020						
Relative humidity (%)	72.9	69.2	77.6	70.7	66.4	69.0
Rainfall (mm)	39.0	5.6	8.8	10.6	9.2	4.6
Temperature (°C)	8.9	7.2	5.3	5.9	**11.3**	11.1
2021						
Relative humidity (%)	69.1	83.1	75.2	72.7	75.1	73.3
Rainfall (mm)	5.1	20.0	22.6	26.0	20.6	10.4
Temperature (°C)	3.6	3.3	3.9	6.3	7.3	**11.3**

For these reasons, in the first year of the study (2019), the earliest blossoming started for 'Jojo' cultivar on 28 March, followed by 'Tegera' and 'Elena' (29 March), and the latest (5 April) for 'Kystendilska'. The end of full blossoming was from April 13 to 19 for 'Stanley', 'Hanita' and 'Kyustendilska' plum cultivars (Figure 2).

In 2020, the earliest blossoming period began for 'Jojo' (7 April.) and the latest (15 April) for 'Kystendilska', but this is 10 days later than in 2019. The latest date for the end of blossoming period for 'Kyustendilska', 'Katinka' and 'Hanita' cultivars was reported on 30 April.

In 2021, blossoming period began for 'Jojo' and 'Tegera' (9 April), whereas the latest date for the beginning was registered for 'Kystendilska' (23 April). The latest date for the end of blossoming was registered for 'Kystendilska' (06 May) and 'Katinka' (02 May) reaching the month of May. In 2020, the duration of blossoming stage was about 3 days shorter than 2019. On average, the blossoming period is about 15 days, ac the longest blossoming period was registered for ‘Mirabelle de Nancy’. The high temperature of spring causes a shorter flowering period and the "full flowering" phenophase lasts only a few days.

‘Jojo’ is defined as the earliest blossoming of the group of German cultivars, followed by ‘Tegera’ and ‘Hanita’. ‘Kyutendilska’ completed its blossoming phase in 2019 – 05 April; in 2020 - 15 April; 2021 - 06 May. This has been investigated and confirmed in our other studies as well (Stefanova et al., 2017; Stefanova, 2019).

Milatovic et al. (2017), the average time of flowering of tested cultivars was in the first half of April, and the average duration of flowering varied from 7.8 to 11.3 days. The average time of maturation ranged from July, 17 (‘Valerija’) to July, 31 (‘Hanita’).

For each year, within 10-11 days, there was a complete overlap of the blossoming of all investigated cultivars. In 2019, this was the period from 02 to 13 April; in 2020 from 10 to 20 April; in 2021 from 16 to 26 April. This indicator ensures good mutual pollination of the cultivars and favours the harvest.

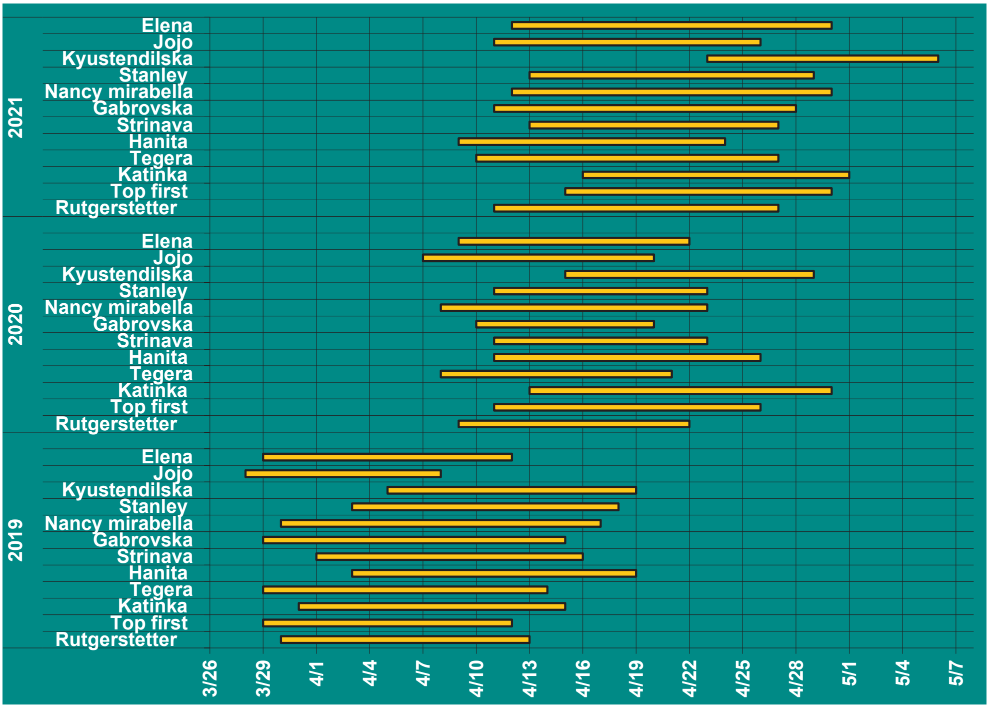

Figure 2. Blossoming phenogram (2019-2021)

The studied plum cultivars cover a harvest period from mid-July (‘Rutgerstetter’) to the end of September (‘Elena’ and ‘Kyustendilska’ cultivars). For the ‘Stanley’ cultivar, in most cases the fruits reach ripening stage at the end of August, beginning of September. In the second half of August and the beginning of September, the average daily temperatures in 2021 were higher and the low amounts of precipitation, compared to the base 20 year period, caused an earlier onset of ripening stage by about 5-6 days, compared to previous years (Figure 3).

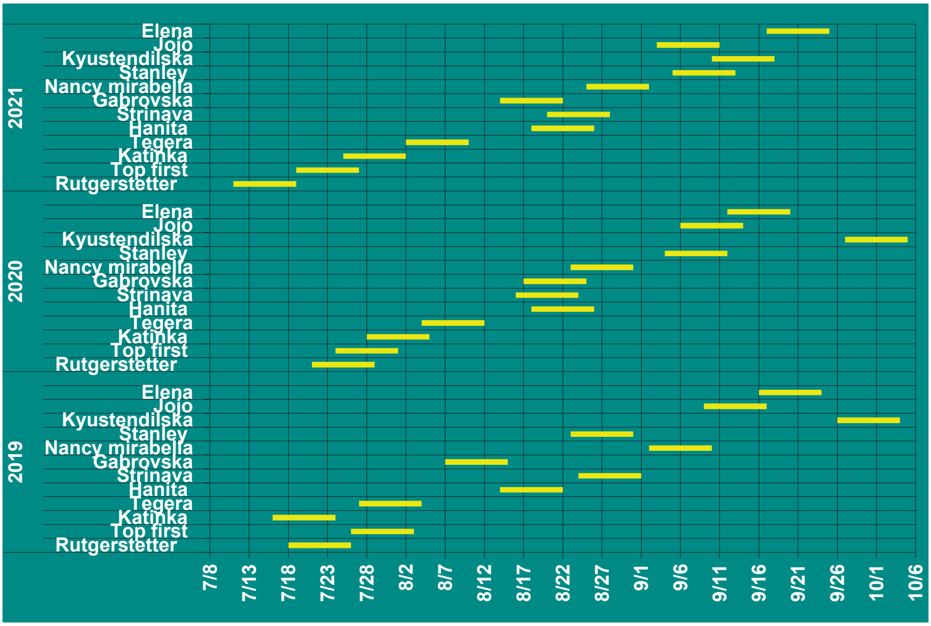

Figure 3. Ripening Phenogram (2019-2021)

Directly related to the power of flowering and the duration of flowering is the realized yield. Agro-ecological factors and their variability affected flowering, by later onset of phenophases, but posed a risk of late spring frosts. They are most sensitive in the fruit set phase. Certain climatic conditions also affect the attack of diseases. Early brown rot compromised the harvest in 2019, yields (measured real and objective) were the lowest for the study period. The highest yields were registered in 2021, as a result of the favourable course of blossoming, good pollination and the formation of a large percentage of fruit-set. Heavy rains in June and July (Figure 1) allowed the fruits of most cultivars to ripen. The highest yields were gathered from 'Kyustendilska' (31 kg), 'Stanley' (26 kg), 'Tegera' (26 kg). These conditions, without prerequisites for the development of economically important fungal diseases, allowed the cultivars to reach their biological potential, with the exception of 'Strinava' and 'Jojo', which are susceptible to drought and remain with the lowest yields in 2021 (Table 2).

Table 2. Average yield per a tree by cultivars (2019-2021)

Average yield per a tree (kg)	2019	2020	2021
Rutgerstetter	3.0	4.2	7.0
Top first	4.3	3.0	5.0
Katinka	17.0	13.5	19.0
Tegera	12.0	14.3	26.0
Hanita	7.5	8.0	19.0
Strinava	19.5	23.4	8.0
Gabrovska	13.0	19.2	17.0
Nancy mirabella	22.5	26.5	32.0
Stanley	14.0	22.3	26.0
Kyustendilska	5.3	11.0	31.0
Jojo	24.0	25.0	6.0
Elena	14.5	12.7	17.0
St. Dev.	*7.000*	*8.015*	*9.678*
CV (%)	*53.64*	*52.53*	*54.52*

CONCLUSIONS

The earliest blossoming of plum cultivars for the conditions of the Troyan region occurred at the beginning of April in 2019. Each subsequent year, the permanent increase in temperatures above 10°C was delayed by 10 days.

The ‘Jojo’ cultivar is determined as the earliest blossoming of the German cultivar group, followed by ‘Tegera’. ‘Kyustendilska’, ‘Katinka’ and ‘Hanita’ cultivars had later blossoming period. The blossoming period for ‘Kyustendilska’ both began and ended at the latest time.

Every year, within 10-11 days, there was a complete overlap of the blossoming of all the studied cultivars. It is the longest for the ‘Mirabella du Nancy’ cultivar (17-18 days).

In 2020, the average blossoming period for all cultivars was about 3 days shorter than in 2019 and 2021.

The drier conditions in 2021 caused an earlier ripening of the fruits by about 5-6 days, compared to the previous years.

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INCREASING THE EFFICIENCY OF PLUM BREEDING IN FAMILY ORCHARDS BY INTRODUCING INTO THE VARIETAL ASSORTMENT OF SOME UNIVERSAL VARIETIES WITH VALUABLE PHYSICOCHEMICAL PROPERTIES

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Abstract

The research was carried out in an orchard from Cenad village, Timis County, throughout 2021, and compared five recently introduced varieties (Topend Plus®, Topfive®, TopHit , Toptaste®, Amers) with a control variety (President). Some physical and chemical characteristics of the fruits (major diameter, minor diameter, length, weight, total soluble solids, carbohydrate content, pH, and vitamin C) as well as plum production were monitored and measured. The plum components and economic indicators differ significantly between the varieties evaluated. In the majority of the examined indicators, all five newly introduced varieties outperformed the control, indicating the necessity to change the plum varietal assortment not only to satisfy market demands, but also to meet the suitability for industrialization and obtaining jams, juices or compotes, an important criterion given the net downward trend in demand for alcoholic products derived from plums. Economic indicators must be considered for fruit breeding success, particularly in light of current market trends caused by increases in labor costs, fertilizers or pesticides.

***Key words**: plum trees, varieties, weight, firmness, yield, assortment, orchards.*

INTRODUCTION

The great adaptability to different climate and soil conditions has made the plum to grow and produce spontaneously or cultivated, with the area of spread and the variety of varieties being practically limitless (Käthner et al., 2017; Svanes and Johnsen, 2019). The plum tree is found all over the world, particularly in temperate climates of the northern hemisphere (Dimitrova et al., 2021). With around 1.7 million ha and a total production of approximately 13.5 million tons, the species ranks 11th in the world after apples, citrus fruits, bananas, peaches, and pineapples, and second in temperate areas after the apple (Ukar et el., 2022). Asia produces the most plums, followed by Europe, North America, South America, Africa, and Oceania (Afanador-Barajas et al., 2023). China, the United States, Turkey, Argentina, Yugoslavia, and Romania are the world's top producers (Wang et al., 2023). Former Yugoslavia, Romania, Germany, Bulgaria or France, are European major producers countries. In this context, Romania has become the Balkan and European country with the highest plum production, as well as a major exporter of fresh or dehydrated fruit (Zagrai et al., 2022).

The large number of plum varieties, which exceeded 2,000 at the beginning of the twentieth century, is the result of an ancient culture whose beginnings are lost in antiquity (Suranyi, 2019). However, in recent years, a severe decrease in the cultivated areas with plum trees has been noticed, even though there are currently programmes that support the establishment of new orchards (Kaufmane et al., 2019).

Romanian fruit growers have recently turned their attention to crops that are more profitable (apricot, peach, cherry) or easier to manage (hazelnut, walnut), even in good and highly favourable places for plum orchards (Gitea et al., 2019). Nonetheless, due to its adaptability and economic value, plum culture will undoubtedly retake its former position in Romania's fruit-tree crop hierarchy.

The plum varietal assortment in western Romanian orchards is relatively limited to two varieties: Stanley and Anna Spath. This assortment has recently begun to be slightly diversified by the introduction of new cultivars with high economic value. This diversification, however, must be of good quality, offering truly valuable cultivars compatible with the pedoclimatic conditions of each orchard location (Woznicki et al., 2019).
Plums are known to be consumed fresh but also dehydrated (dried plums), and they are used to make jams, marmalade, compotes, jellies, liqueurs, and last but not least the famous Romanian plum liqueur (Varga et al., 2022). Plum blossoms are an important source of honey and provide a spectacular view for entire regions (Hasnain et al., 2023; Fotirić Akšić et al., 2022).
When compared to other fruits, plums have the highest nutritional value. Plums have diuretic, laxative, depurative, nerve stimulant, and liver decongestant properties, with a sugar content of 16-20% (malic acid and in small amounts citric and benzoic acids) (Lin et al., 2023).
Fresh plums contain all of the microelements that humans require for normal life, such as potassium 170 mg%, Ca 12 mg%, Mg 10 mg%, P 18 mg%, Na 1mg%, Fe 0.5 mg%, Cl 1.5 mg%, and so on (Tomić et al., 2022). Among the most representative vitamins are: vitamin C under 4.0 mg%, carotene 0.9 mg%, B 10.83 mg%, B1 0.03 mg%, PP 1.0 mg% and others (Panahirad et al., 2019). As a result, plums are balanced foods in terms of vitamins, minerals, carbohydrates, and other nutrients or regulatory substances of human metabolism (Li et al., 2023). The world trends regarding the improvement of varieties are common, and they propose the preservation of valuable varieties, that respond to the local pedoclimatic and agrotechnical requirements on the one hand and to commercial and consumer ones on the other, with good economic results (Kuchay et al., 2022). The trees must produce abundantly and constantly from the first years, with significant adaptability to the various pedoclimatic zones, be rustic, quite resistant to diseases (Seethapathy et al., 2022).
The fruits must be large (over 40 g), attractively colored, with a pleasant taste, sugar, acidity, and tanoid substances in a well-balanced ratio, and a rich content of vitamins and mineral substances useful for the human body (Park et al., 2021).
The study's objective was to evaluate the physical and chemical parameters of several plum varieties cultivated in the environment of western Romania.

MATERIALS AND METHODS

The research was conducted during the year 2021, in an orchard located in the Cenad area, Timis County. The characteristics of some varieties recently introduced into the area's varietal assortment (Topend Plus®, Topfive®, TopHit, Toptaste®, Amers) were monitored. The observations and determinations were made in comparison to the President variety, which is grown in the area and provides good to very good results.
The study focused on the phenology, physical, and chemical characteristics of the fruits, such as length, width, weight, soluble dry matter, pH, carbohydrate, malic acid, and vitamin C content, fruit firmness, and plum production. BBCH codes were used to evaluate the phenophases of flowering and harvest maturity.
The soluble sugar content was measured with the digital refractometer, the pH was determined using a pH metre, the firmness of the fruits was determined using a penetrometer, and the acidity was determined by titration. The HPLC method was used to separate vitamin C by high-performance liquid chromatography.

Statistical analysis

The XLSTAT software was used to analyse data from experiments designed in accordance with specific statistical designs (by Addinsoft, 2018, Statistical and Data Analysis Solution Version 2018.7.5). P values ($p \leq .05$; $p \leq .01$ $p \leq 0.001$) were used to estimate the source of variation.

RESULTS AND DISCUSSIONS

The recent climatic variability has influenced the success of plum culture in many circumstances, which is why fruit growers have changed their strategy to varieties with later flowering or are experimenting with various methods to induce a phenophase delay of at least a few days (Florea et al., 2019). The investigated varieties flowered later than the control variety, both in terms of phenophase initiation (BBCH 61) and phenophase ending (BBCH 65) (Meier, 2018).

Early flowering varieties are more vulnerable to late spring frosts than later ones. Late frosts have a greater negative impact on production when the climatic incident happens in BBCH 65, as compared to BBCH 61. Toptaste® and Topend Plus® varieties reached the BBCH 65 phenophase 6 and 5 days later, respectively, than the control variety. TopHit and Amers varieties, which reached the BBCH 65 stage at the same time as the President variety, were at the opposite pole (Table 1).

Table 1. Flowering stage code

Variety	Beginning of flowering BBCH 61	Full flowering BBCH 65	Difference to control (days) BBCH 65
Topend Plus®	15.04	21.04	5
Topfive®	13.04	19.04	3
TopHit	9.04	16.04	-
Toptaste®	15.04	22.04	6
Amers	10.04	17.04	1
President (C)	9.04	16.04	-

Table 2. Plums maturity stage - BBCH 87

Variety	August			September			October
	1-10	10-20	20-30	1-10	10-20	20-30	1-10
Topfive®	8.08						
Toptaste®		12.08					
TopHit				7.09			
Amers					12.09		
President (C)					19.09		
Topend Plus®							8.10

Regarding harvest maturity, it is preferable that the chosen assortment be planned over a longer period in order to avoid production peaks, better manage the required labour force, and ensure the consumption and storage of fresh fruit for as long as possible. The Topfive® variety was the first to reach harvesting maturity (BBCH 87) among the varieties studied, followed by the Toptaste® variety 4-5 days later (Table 2). These two varieties ensure early production, which benefits from higher prices at the start of the season, for plums in the first half of August. TopHit reached harvest maturity around 12 days earlier than the control variety in the first decade of September. Topend Plus® was the only variety that achieved harvesting maturity later than the control variety, allowing a late consumption of plums towards the end of the season when prices become competitive again. The chosen assortment ensures that the plum production is distributed over a long period of time, around 60 days, allowing for both an easier distribution of the production and a decrease in the pressure on the processing activities.

Table 3. Plums length and width measurements

Variety	Major diameter (L)		Minor diameter (l)		Difference to control (mm)	
	mm	%	mm	%	L	L
Topend Plus®	45.68	104.48	42.78	104.06	1.96^{ns}	1.67^{ns}
Topfive®	39.22	89.70	36.12	87.86	-4.5^{**}	-4.99^{*}
TopHit	50.92	116.46	48.25	117.36	7.2^{***}	7.14^{***}
Toptaste®	41.89	95.81	38.95	94.74	-1.83^{ns}	-2.16^{*}
Amers	49.74	113.76	44.22	107.56	6.02^{***}	3.11^{***}
President (C)	43.72	100.00	41.11	100.00	-	-

*t significant at $p \leq .05$; **t significant at $p \leq .01$;***t significant at $p \leq .001$

Table 4. Main physical parameters of six plum varieties

Variety	Length		Weight		Difference to control (mm)	
	mm	%	g	%	Length	Weight
Topend Plus®	53.64	103.09	61.12	113.10	1.61 ^{ns}	7.08***
Topfive®	53.11	102.07	57.23	105.90	1.08^{ns}	3.19*
TopHit	61.12	117.47	76.38	141.33	9.09***	22.34***
Toptaste®	50.17	96.42	52.26	96.70	-1.86*	-1.78^{ns}
Amers	54.11	103.99	59.21	109.56	2.08 ^{ns}	5.17**
President (C)	52.03	100.00	54.04	100.00	-	-

*t significant at p ≤. .05; **t significant at p ≤ . 01;***t significant at p ≤. .001

Like other fruits, buyers choose plums mostly based on how they look; the largest plums with the most appealing skin are always in demand. The success of the product's marketing is assured if the physical traits are combined with palatable flavour qualities and a non-sticky pulp. It is desirable that most of the production be valued through direct fresh consumption that ensures immediate income. Therefore, all the researched varieties have large, attractive fruits, with a plus for the TopHit and Topend Plus® varieties, which, compared to the control, have a statistically significant higher fruit weight. The only variety that, both in terms of fruit length and weight, had lower values than the control was Toptaste® (Tables 3, 4). In similar research done on 10 varieties of plum, Tomić et al. (2022) found an average plum weight ranging from 26.9 to 57.9 g. Dimitrova et al. (2017) after the research of fresh fruits of 10 cultivars, found an average shorter length of plums among 33.26 and 47.8 mm.

Table 5. Chemical properties of plums varieties

Variety	Total soluble solids (TSS)		Carbohydrates		Difference to control (mm)	
	(% Brix)	Content	%	Content	Total soluble solids	Carbohydrates
Topend Plus®	19.27	98.52	18.16	98.64	-0.29^{ns}	-0.25^{ns}
Topfive®	19.84	101.43	18.59	100.97	0.28^{ns}	0.18^{ns}
TopHit	19.23	98.31	18.11	98.37	-0.33^{ns}	-0.3^{ns}
Toptaste®	20.09	102.71	18.88	102.55	0.53*	0.47*
Amers	20.11	102.81	18.93	102.82	0.55*	0.52*
President (C)	19.56	100.00	18.41	100.00	-	-

*t significant at p ≤ .05;

Besides appearance, the taste and chemical composition of plums are important, especially when the fruits are processed (Table 5). Total soluble solids were comparable between varieties, ranging from 19.27 to 20.09% Brix. Toptaste® and Amers were the only varieties that outperformed the control with statistically significant differences. Plocharski and Konopacka (2003) found in two Polond plum varieties a lower total soluble solids amount between 11.8 and 16.9%.

Plum varieties had very similar carbohydrate content, with limits ranging from 18.11 to 19.93%; the differences in carbohydrate content between the Amer and Toptaste® varieties compared to the control were also statistically significant.

In addition to the dry matter and carbohydrate content, the pH, malic acid content, and vitamin C content are important parameters that contribute to balanced overall plum content (Table 6).

The ph level for all varieties was generally close, with limits ranging from 3.55 for the control variety to 3.86 for the Amers variety. It should be noted that the pH recorded slightly significant values for the control variety in all varieties investigated. Larger and higher limits of Vitamin C (4-11 mg/100 g) were found in Poland plums by Walkowiak-Tomczak (2008).

Table 6. Plums pH and malic acid content (% or mg/100 g fresh matter)

Variety	pH	malic acid (% or mg/100 g fresh matter)	Vitamin C (mg/100 g)
Topend Plus®	3.75	0.74	3.94
Topfive®	3.62	0.67	3.88
TopHit	3.57	0.63	3.65
Toptaste®	3.59	0.66	3.73
Amers	3.86	0.62	3.57
President (C)	3.55	0.71	3.91

Table 7. Firmness in plums variety

Variety	Firmness (kg/cm^2)	Relative value (%)	Difference to control
Topend Plus®	2.42	109.50	0.21*
Topfive®	1.98	89.59	-0.23*
TopHit	1.85	83.71	-0.36*
Toptaste®	1.94	87.78	-0.27*
Amers	2.09	94.57	-0.12^{ns}
President (C)	2.21	100.00	-
*t significant at p ≤. .05;			

The malic acid content was also within normal limits, with close relative values between the varieties, ranging between 0.62 for the Amers and 0.74 for the Topend Plus® variety.

Plums are known to be vitamin C-rich fruits, with the content ranging between 3.57 mg/100 g for the Amers variety and 3.94 mg/100 g for the Topend Plus® variety.

The only variety that recorded higher values than the control for vitamin C content was the Topend Plus® variety.

Table 8. Plums production parameters

Variety	Plum production kg/tree	kg/ha	%	Difference to control (kg/ha)
Topend Plus®	32.3	20187.5	110.6	1937.5***
Topfive®	28.2	17625.0	96.5	-625.0*
TopHit	29.4	18375.0	100.7	125.0^{ns}
Toptaste®	26.5	16562.2	90.7	-1682.8**
Amers	31.6	19750.0	108.2	1500.0**
President (C)	29.2	18250.0	100.0	-
*t significant at p ≤. .05; **t significant at p ≤ .01;***t significant at p ≤. .001				

Fruit firmness is an essential variable that impacts the shelf life of the product, transport resistance, and handling (Table 7). Firmness values ranged from 1.85 for the TopHit variety to 2.42 for the Topend Plus® variety, which was also the only variety with significantly better firmness than the control variety. Topfive®, TopHit®, Toptaste®, and Amers varieties are not considered less popular despite having lower firmness values than the control.

Finally, the plum production obtained is the most important indicator that has a significant impact on the economic results and profit of each orchard. The researched varieties produced favourable results, with yields ranging from 26.5 kg/tree for the Toptaste® variety to 32.3 kg/tree for the Topend Plus® variety, yielding 16562.2 kg/hectare and 20187.5 kg/hectare, respectively (Table 8).

Although the Topfive® and Toptaste® varieties have early fruit ripening, they have reached satisfactory levels of production per hectare, which, coupled with higher prices since the beginning of the consumption season, make these varieties attractive variants within the assortment of varietals. Topend Plus® and Amers, the most productive varieties, were the only ones that outperformed the control with statistically significant differences. These are also the latest varieties, ensuring an extension of the plum consumption season and, as a result, off-season prices.

CONCLUSIONS

Plum culture is very common in Romania, from the plains to the sub-Carpathian areas and even to the highlands. However, for unknown reasons, there has been a significant decrease in the ratio of newly established plum orchards to those of other fruit tree species in the past few years. With the development of new plantations of other fruit tree species, like hazelnut, walnut, and cherry, areas planted with plum have been limited in the western part of the country.

In the west of the country, the plum varietal assortment is relatively small, with the Stanley and Anna Spath varieties predominating in a significant proportion. This assortment no longer meets the consumer's current demand for more diverse products, ranging from fresh plums to dehydrated plum consumption, juices, nectar, and other processed products. As a result, it is necessary to expand the varietal assortment by introducing valuable varieties with clearly different appearance and quality characteristics that are suitable for multiple applications. The new varieties introduced into the assortment must be adaptable to the pedoclimatic resources of the area while also being resistant to diseases and pests, requiring fewer treatments, or even being cultivated in order to achieve financial efficiency, in an organic system that has been successfully implemented in Romania in recent years. Climate change has caused significant production losses in recent years, including in plum orchards; therefore, the later the varieties bloom, the less likely they will be destroyed by late spring frosts. Toptaste® and Topend Plus® are recommended in this regard because they bloom 5-6 days later than the control. The studied varieties responded favorably to the natural conditions of the cultivation area, yielding positive results for the commercial quality standards, the physico-chemical composition, and plum production.

Due to the distribution of fruit maturity over a period of about 60 days, the plum assortment chosen ensures a long period of fresh fruit and benefits from good prices from the beginning (Topfive® and Toptaste®) to the end of the season. All five investigated varieties are also suitable for processing, particularly as juices and nectar, due to their sugar, acidity, pH, and malic acid content.

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APRICOT RESPONSE TO RADICULAR AND FOLIAR APPLICATION OF FERTILIZERS UNDER DOBROGEA CONDITIONS

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Abstract

Drought and heat stress are significant factors limiting fruit crop yield in arid conditions. Foliar fertilization is a common practice of supplying fruit crop production with mineral nutrients, especially under limited soil nutrient availability conditions. Nutrient management is a determining element of the technology in fruit quality. In this study we aimed to evaluate the impact of a the radicular (NPK+S) and foliar (Cropmax) fertilizer on fruit quality parameters in an apricot orchard during 2019 -2021 period. According to our results, the weight of the fruits increased by 30% in the b1 treatment (radicular fertilizer with NPK+S) and 34% in the b2 treatment (radicular and foliar fertilizer with Cropmax) at Olimp cultivar and by 40% in the b1 treatment and 53% in the b2 treatment at Goldrich cultivar in the three years of study compared to the control treatment. In conclusion, the fruit quality can be improved in apricot orchard using of the radicular and foliar fertilizer.

***Key words**: climate conditions, fertilization, fruit size, Prunus armeniaca, weight.*

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is a mesotonic diploid fruit tree species belonging to the *Rosaceae* family and originated from Central Asia (Janick, 2005). Globally, the apricot orchard systems are shifting from medium-low planting densities (600-750 trees/ha) to high-density (1000-1250 trees/ha) plantings (Giovannini et al., 2010).

Essential plant nutrients are mainly applied to soil and plant foliage for achieving maximum economic yields (Fageria et al., 2009). The fertilization is a great way to maintain the health and vigor of trees. Fruit tree crops are agricultural commodities of great biological and economical importance, and therefore, precise knowledge of treatments that boost fruit production and quality, is of great importance. The quality of fruits, except for genetic characteristics, are significantly influenced by cultivation techniques. Fertilization is one of these practices. Nowadays foliar fertilization has become a basic management tool in the intensive orchards. Perennial fruit trees are frequently exposed to various abiotic stresses during their lifetime that limit crop yield and quality. Foliar fertilizers have an interesting potential to improve fruit quality, with relatively low costs and low environmental impact (Csihon et al., 2021). Application of sprays supplies nutrients to plants more rapidly than the soil fertilization (Tagliavini et al., 2002; Nagy et al., 2012; 2019).

The benefits of biostimulators on fruit quality are also confirmed by other researchers (Hudina et al., 2003; Basak & Mikos-Bielak, 2008; Nagy et al., 2019; Csihon et al., 2013; 2021).

The aim of this work was to provide data on the effect of "Cropmax" foliar biostimulator on the fruit quality in an apricot orchard.

MATERIALS AND METHODS

Experimental site. The experimental lot is located in Valu lui Traian commune, Constanta district, Dobrogea region, Romania. The climate is semiarid, with a climatic water deficit (WD), ranging from approximately -400 mm on the Black Sea coast to -320 mm (Paltineanu et al., 2007). The soil is a calcareous chernozem with clayey texture and alkaline soil pH that has good soil structure (0-60 cm deep, with 27-32% w/w clay content,

1.6-2.8% w/w humus content, 1.5-6.8% w/w carbonate content), while in the unstructured subsoil, the humus content is less than 1% w/w and carbonates from 9 to 14% w/w; the slope of the land is between 2.0 and 2.5% (Paltineanu et al., 2011).

The apricot orchard was planted in spring of 2012. Trees were grafted on Constanța 14 rootstock and designed with spacing of 4 x 2.5 meter with north-south row orientation. The canopy shape was a classic vase with the height of 2.5 m.

The experiment design was based on the split-plot method with three treatments: **1)** radicular fertilizer with NPK+S, 15: 15: 15 + 12.8; **2)** radicular and foliar fertilizer (Cropmax); **3)** control, each one containing four replicates. The soil management system is represented by clean cultivation both between tree rows and in the row. Plant protection refers to the principles of integrated pest management.

The climatic data: solar radiation, air temperature, relative humidity, wind speed at the height of 2 m, precipitation (P) and Penman-Monteith reference evapotranspiration have been recorded by an automatic weather station (iMetos, IMT 300, Pessl Instruments, Austria) by a 1-h step. These data have been periodically processed as diurnal means and used in calculations.

Applied treatments. The main objective of this study was to evaluate the impact of radicular and foliar fertilizer on the fruit quality in an apricot orchard. The trial consisted of three treatments (control, radicular fertilizer and radicular+foliar fertilizer). The first treatment with foliar fertilizer was applied in full bloom in 2019, 2020 and 2021, then the treatments were repeated 3 times (Table 1). Biostimulant treatment was applied to apricot orchard in the vegetation phases with 0.5 L/ha dosage (Figure 1). Every spring, in the apricot orchard was applied to the soil the complex NPK fertilizer enriched with easily absorbable S (15: 15: 15+12.8), 500 kg/ha, respectively. Each treatment was applied to 16 trees.

Table 1. Foliar fertilizer application moments (2019-2021)

2019	2020	2021
01.04.2019	08.04.2020	19.04.2021
13.05.2019	17.05.2020	11.05.2021
29.05.2019	31.05.2020	09.06.2021
12.06.2019	17.06.2020	25.06.2021

Figure 1. Foliar fertilizer applied at apricot orchard

Applied materials. The apricot tree (*Prunus armeniaca*) has been selected for this study as it is one of the most cultivated fruit tree species worldwide, especially in the warm temperate climate regions. Olimp is an apricot cultivar obtained at Research Station for Fruit Growing (RSFG) Baneasa and patented in 1994 (Figure 2). Goldrich is a cultivar of apricot of American origin (Figure 3).

Figure 2. Olimp cultivar

Figure 3. Goldrich cultivar

NPK complex fertilizers, with a balanced formula in the content of nitrogen (N), phosphorus (P), potassium (K), guarantee a fertilization rich in nutrients with direct results on increases in the quality and quantity of fruit products. Cropmax is a complex super concentrate nutrient for foliar fertilization, which is 100% natural, produced by Farming Holland BV. Cropmax fertilizer presents the following chemical composition: growth plant stimulator (auxines, cytokinins, gibberelins), organic amino acids, vegetal vitamins, vegetal enzymes, macroelements: N - 0.2%, P - 0.4% , K - 0.02%, Fe - 220 mg/L, Mg - 550 mg/L, Zn - 49 mg/L, Mn - 54 mg/L, Cu - 35 mg/L, other elements: B, Ca, Mo, Co, Ni - 10 mg. Being a natural product, Cropmax is well absorbed by leaves and stems. Influence on photosynthesis leads to the increase of carbohydratesamount. Also, are reduced metabolic deficiencies.

Assessed parameters. Each year was tested average samples of 15 fruits/treatment. Fruit growth was monitored by measuring longitudinal and transversal fruit diameter and fruit height after harvest. The measurements were performed using a metric digital caliper (Insize Co., Ltd. China). The average weight of a fruit was determined by weighing 10 fruits/treatment and dividing by the number of weighed fruits. The weighing of the fruit was performed with a precision balance (Kern & Sohn GmbH, Germany). Fruits of Olimp were harvested between the 8th to the 9th of July in 2019, from 16th to the 17th of July in 2020 and 25th to the 26th of July in 2021. Fruits of Goldrich were harvested between the 26th to the 27th of June in 2019, from 1st to the 2nd of July in 2020 and 8th to the 9th of July in 2021.

Data analyses. SPSS 14.0 software and Microsoft Office Excel were used for the analysis of variance and various calculations for fruit quality properties. Different letters in the graphs indicate significant differences for the probability (P) ≤ 0.05 according to Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Climate conditions. In the experimental period, in the growing season, the climate conditions are characterized by mean annual values of temperature and precipitation of 19.5 °C and 213.8 mm, respectively, and precipitation is not uniformly distributed across the growing season. The reference evapotranspiration was 717.0 mm with mean values of 134.0, 150.0 and 134.7 mm month^{-1} during summer months: June, July and August. The climatic water deficit (WD= P-(PM-ET0)) is high in the summertime (-79 mm in June, -124.3 mm in July and -125.0 mm in August) and its sum for growing season is -500.2 mm (Septar et al., 2022). The mean value of yearly and monthly climate data during the growing season in the experimental period is shown in the Figure 4 (a, b) and Figure 5 (a, b).
The period of experiment (2019-2021) was considered as a relatively normal period, with monthly temperature means of 22.3°C in June, 24.0°C in July and 23.9°C in August, respectively.

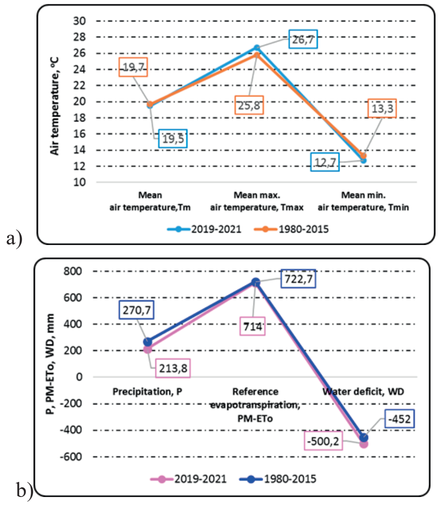

Figure 4. The mean values of annual air temperature, maximum air temperature, minimum air temperature (a), precipitation, reference evapotranspiration and water deficit (b) during the growing season in the 2019-2021experimental period versus the long-term, 1980-2015, Valu lui Traian, Romania

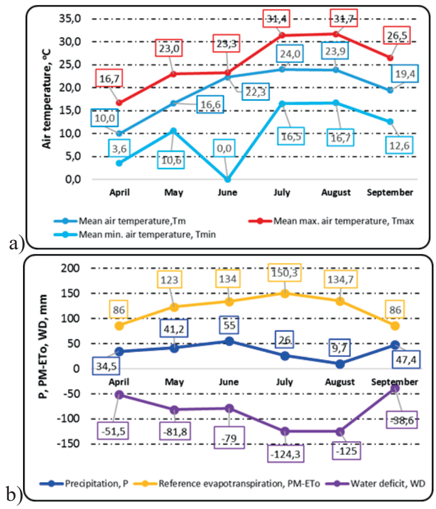

Figure 5. The mean value of monthly air temperature, maximum air temperature, minimum air temperature (a), precipitation, reference evapotranspiration and water deficit (b) during the growing season in the 2019-2021 experimental period versus the long-term, 1980-2015, Valu lui Traian, Romania

Biometric measurements and weight of apricot fruits. After harvesting, in the laboratory, biometric measurements and fruit weighing were performed. The presented values represent average values of the three years of study.

In the Olimp cultivar, the fruits had a longitudinal diameter of 45.98 mm to 54.14 mm, with the largest longitudinal diameter in the b2 treatment. There were significant differences among the studied treatments on fruit longitudinal diameter as indicated by different letters according to probability (P) ≤ 0.05 according to Duncan's multiple range test, Figure 6a. The transversal diameter of the fruits had the same trend. The lowest value was obtained in treatment b3, 41.5 mm and the highest value in treatment b2, 47.3 mm, respectively. Significant differences between the studied treatments in terms of fruit transverse diameter are shown in Figure 6b. The apricot fruits had a height of 53.8 mm to 60.5 mm. Figure 6c shows significant difference between the fertilizer treatments and control treatment regarding the fruits' height.

The fruit weight determined on the fruits of the studied treatments had the same trend. The highest value was recorded in treatment b2, 71.0 g respectively, and the lowest value in treatment b3, 52.9 g. Figure 6d shows significant difference between the fertilizer treatments and control treatment regarding the fruits weight.

In the Goldrich cultivar, fruits had a longitudinal diameter of 46.8 mm to 56.8 mm, with the largest longitudinal diameter in the b2 treatment. Figure 7a shows significant differences between the studied treatments as indicated by different letters according to probability (P) ≤ 0.05 according to Duncan's multiple range test. The transverse diameter had the same trend. The highest value was obtained in treatment b2, 51.0 mm, Figure 7b. Fruit height of the Goldrich cultivar ranged from 50.4 mm to 60.2 mm. Figure 7c shows significant differences between the studied treatments in terms of fruit height. The fruit weight determined on the fruits of the studied treatments varied between 87.1 g in the b2 treatment and 56.9 g in the b3 treatment. Significant differences between the studied treatments are shown in Figure 7d.

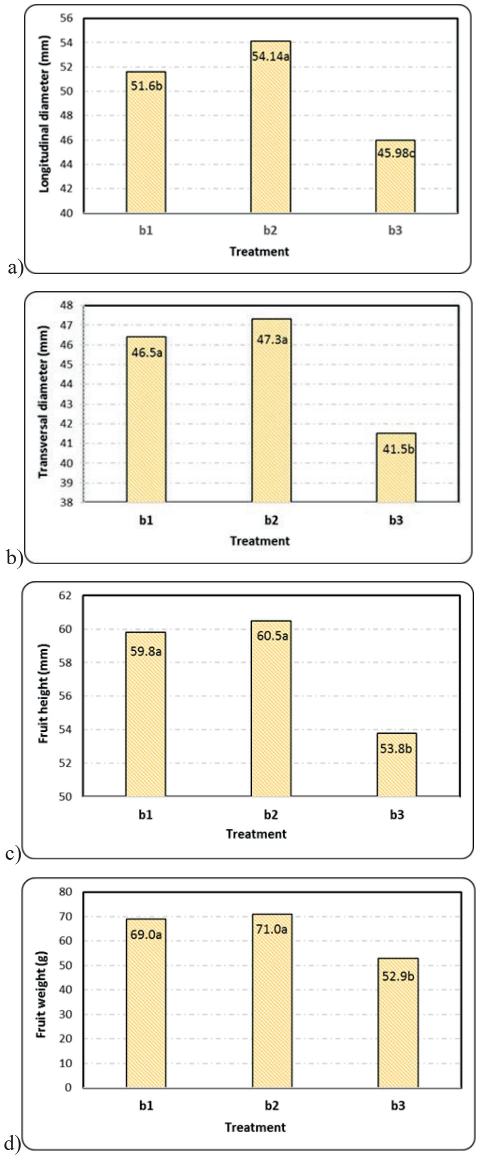

Figure 6. Longitudinal diameter (a), transversal diameter (b), fruit height (c) and fruit weight (d) of the fruits to Olimp cultivar, 2019-2021

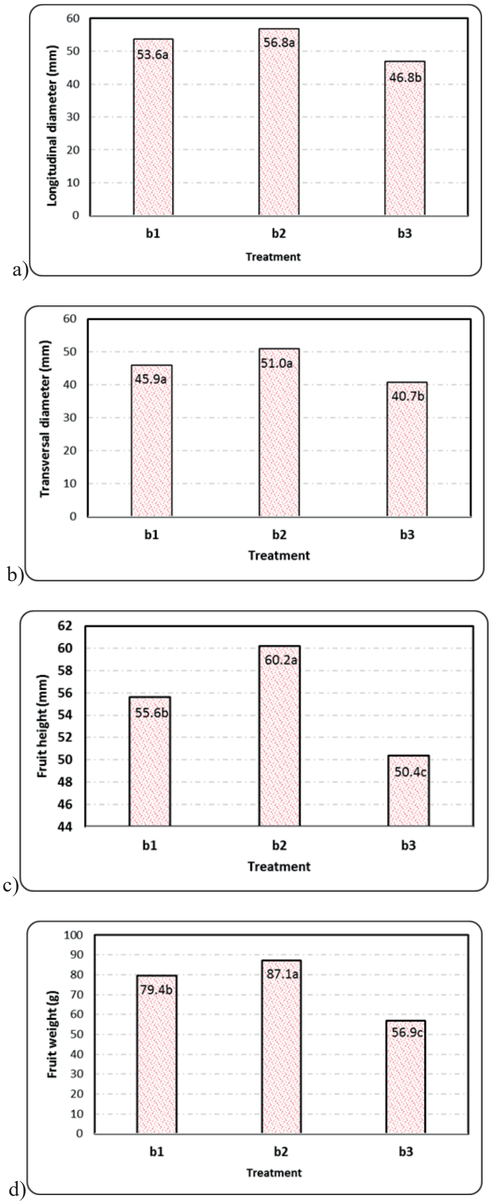

Figure 7. Longitudinal diameter (a), transversal diameter (b), fruit height (c) and fruit weight (d) of the fruits to Goldrich cultivar, 2019-2021

CONCLUSIONS

The obtained results show that the combined use of radicular and foliar fertilization contributes to the improvement of fruit quality.

In Olimp and Goldrich cultivars, fruit weight was higher compared to the control treatment using radicular fertilization with NPK+S and foliar fertilization with Cropmax.

Application of bio-stimulator treatments resulted in improvement of all biometric indicators for apricot fruits.

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PAWPAW FRUIT (*ASIMINA TRILOBA* (L.) DUNAL). PROCESSING AND NUTRACEUTICAL VALUE

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Abstract

Asimina triloba (L.) Dunal, or pawpaw, is the only temperate plant species that belongs to the Annonaceae family. Is a native North American fruit species from Florida to South Canada. In Romania, the first pawpaw plants were brought in Transylvania from North America at the beginning of the 20th Century, in 1926 by Suciu family from Alba County. They were locally cultivated and remained unknown in the rest of country. Only after 2000, have begun to be studied at the Faculty of Horticulture, in Bucharest. Regarding nutritional value, asimina is comparable to apple, banana, orange, peach and grape since it is high nutritionally rich fruit with high levels of minerals, vitamins and antioxidant compounds. The color of the fruit changes from white-cream at bright yellow to shades of orange. The flavor of ripe pawpaw fruit resembles a combination of banana, mango and pineapple. Pawpaw fruit are best eaten fresh when fully ripe but the intense tropical flavor may be useful for preparation of food products such as: ice cream, smoothie, candy, juices, cakes and others.

Key words: *northen banana, products, minerals, vitamins.*

INTRODUCTION

Asimina triloba (L.) Dunal, or pawpaw, is the only temperate plant species that belongs to the *Annonaceae* family (Padmanabhan & Paliyath, 2016; Zhang Lin, 2016) the tropical custard-apple family and is the largest tree fruit native to the United States (Darrow, 1975; Desmond, 1996). All but one of the 130 genera of the *Annonaceae* family thrives in the tropical region. Only the genus *Asimina* grows in the temperate climate zone (Callaway, 1993), specifically the USDA growing zone 5 (Brannan et al., 2012; Pomper et al., 1999).

Is a native North American fruit species including nine species of *Asimina* (Padmanabhan & Paliyath, 2016) grows on the eastern part of the continent, from Florida to South Canada (Stan et al., 2022). In the south-eastern part of Florida and Georgia State, there are eight other members of *Asimina* genus: *Asimina incarna* (flag paw-paw), *Asimina longifolia, Asimina obovata, Asimina parviflora* (dwarf paw-paw), *Asimina pygmaea*, *Asimina reticulata, Asimina tetramera* (oposum paw-paw), *Asimina × nashii* (Callaway, 1993; Stan et al., 2022; Stănică et al., 2008).

About 70 varieties of *Asimina* have been cited in the literature, but only 40 are currently commercially available (Stănică et al., 2008).

Asimina triloba is a species with high frost resistance, surviving at -25 to -30°C, well adapted to different soil types, preferring loose, well-drained soils, with a neutral or slightly acidic pH. Also, it has a resistance to diseases and pests, being easy to grow in the organic system (Stănică, 2012; Tabacu et al., 2020).

Is reported to be the largest tree fruit native to the United States because the pawpaw fruit, which can grow up to 1 kg by weight (Darrow, 1975; Zhang Lin, 2016).

In 1541, was written the first documentation about *Asimina triloba*. In the present, despite its long history, it is still a mysterious fruit that is not commonly recognized.

A sensory study of tropical fruits shows that less than 10% of consumers who liked better the pawpaw taste could identify it correctly from other tropical fruits (Brannan et al., 2012; Zhang Lin, 2016).

In Romania, the first *Asimina* plants arrived in Transylvania from North America at the beginning of the 20th Century, in 1926, when in Pianu Nou, Alba County, Ioan Suciu family obtained plants from some seeds brought from Ohio State (Cepoiu et al., 2004; Stănică, 2002; Stănică, 2012).

They were locally cultivated and remained unknown in the rest of country (Dănăilă et al., 2004; Stan et al., 2022).

Only after 2000, at the Faculty of Horticulture in Bucharest, was a scientific evaluation of this interesting species started with the goal of studying the propagation techniques, orchard management and its behaviour under Romanian conditions (Cepoiu et al., 2004; Stan et al., 2022; Stănică & Cepoiu, 2003; Stănică et al., 2004; Stănică et al., 2008; Stănică, 2012).

Other *Asimina triloba* genotypes are currently found in Romania in the 'Dimitrie Brândză' Botanical Garden of the University of Bucharest and other sites of the city, Geoagiu (Hunedoara County), Simeria, Baia Mare (Cepoiu et al., 2004; Tabacu et al., 2020).

ABOUT *ASIMINA TRILOBA* FRUIT

Usually, the pawapaw fruits is highly perishable and only available for purchase in local markets or from private gardeners' backyards.

The promotion of pawpaw to standardized markets is relatively difficult for it has weaknesses, due to perishability (Zhang Lin, 2016).

The shelf life of a ripened fruit stored at room temperature is 2 to 3 days, but with refrigeration [4°C (39.2°F)], fruit can be held up to 3 weeks while maintaining good eating quality (Layne, 1996; Templeton et al., 2003). Though the fruit is still at the edible stage, after the color of cut pawpaw pulp turning in dark-brown, its appearance is not favorable and for this reason impact consumers' perceptions of quality and freshness (Boyd, 2015; Zhang Lin, 2016).

During the growing season, the pawpaw has a whitish to light-green color. The color of the pulp changes from creamy white through bright yellow to shades of orange that turns to brown at maturity (Brannan et al., 2012; Layne, 1996; Levine et al., 2015; Peterson, 2003; Pomper & Layne, 2005; Wood & Peterson, 1999; Zhang Lin, 2016).

Pawpaw fruit has a sweet and sour taste, the flavor of ripe pawpaw fruit resembles a combination of banana (*Musa* × *paradisiaca*), mango (*Mangifera indica*), and pineapple (*Ananas comosus*); however, flavor varies among varieties, with some fruit displaying more complex flavor profiles (Desmond, 1996; Kobayashi et al., 2008; McGrath & Karahadian, 1994b; Nam et al., 2018; Padmanabhan & Paliyath, 2016; Pomper & Layne, 2005; Stan et al., 2022) and the soft flesh surrounds two rows of large bean-shaped dark brown seeds. The skin of pawpaw should not be eaten. (Brannan et al., 2012; Wood & Peterson, 1999; Zhang Lin, 2016).

Although pawpaw is sometimes confused with papaya (*Carica papaya*), but it is an entirely different species (Levine et al., 2015). Papaya is a tropical plant grown in tropical regions, but pawpaw can grow well in tropical regions as well as in humid microthermal climates (Padmanabhan & Paliyath, 2016; Stan et al., 2022).

In the Figure 1 we found some health benefits of pawpaw fruits.

NUTRITIONAL COMPOSITION AND HEALTH BENEFITS

Pawpaw is a nutritionally rich fruit with high levels of antioxidant compounds, regarding nutritional value. The pawpaw antioxidant content is similar to values for strawberry and orange, and is almost ten times higher than values for banana and apple (Nam & Jang, 2018; Pellegrini et al., 2003).

Brannan et al. (2015), reported that pawpaw fruit contains a large amount of procyanidins, which have antioxidant effects, and Kobayashi et al. (2008), demonstrated that pawpaw fruit exhibits antioxidant activity (Nam et al., 2018).

Pawpaw is a nutritionally superior fruit, being a good source of some vitamins, minerals, and amino acids than in apple (*Malus sylvestris* var. domestica), grape (*Vitis* spp.), and peach (*Prunus persica*) (Jones & Layne, 1997;

Peterson et al., 1982; Templeton et al., 2003). Are high in vitamins such as vitamin C, niacin, protein and minerals, amino acids and they can be considered an excellent source of potassium, calcium, phosphorus, iron and magnesium, all very important micronutrients that are often lacking in the diets of children and seniors, along with unique taste, make it an interesting alternative to the most commonly consumed fruits (Galli et al., 2007; Stan et al., 2022; Templeton et al., 2003).

Figure 1. Health benefits of pawpaw - Source: Original

A pawpaw's caloric content is composed of about 13.5% fat, in contrast with apples (5.5% fat) and bananas (4.7% fat) (Jones & Layne, 1999). Because other fruit purees have been successfully used to replace fat in baked products (Charlton & Sawyer-Morse, 1996; Swanson & Munsayac, 1999), the composition of the pawpaw fruit makes it a unique and realistic candidate as a fat-reducing agent in baked goods (Duffrin et al., 2001).

The general nutritional value data of pawpaw are presented in the Table 1.

Table 1. Fruit nutritional value of pawpaw

Nutritional value (per 100 g)				Vitamins (per 100 g)				Minerals (per 100 g)			
	[1]	[2]	[3]; [4]; [5]		[1]	[2]	[3]; [4]; [5]		[1]	[2]	[3]; [4]; [5]
Energy (Kcal)	85	84	80	**Vitamin A (IU)**		82	1	**Calcium (Ca)/(mg)**	13	8	7.9
Moisture (g)	74.5	79.1	75.3	**Thiamine (B1)/(mg)**			0.8	**Iron (Fe)/(mg)**	0.2	0.3	56
Carbohydrates (g)	23.8	18.6	18.8	**Riboflavin (B2) (mg)**			6	**Magnesium (Mg)/(mg)**		10	35.9
Lipid (g)	0.6	0.4	1.2	**Niacin (B3)/(mg)**			6.5	**Manganase (Mn)/(mg)**			74.3
Protein (g)	0.7	1.5	1.2	**Vitamin C (mg)**	24.01	1.0	30.5	**Phosphorus (P)/(mg)**			5.9
Ash (g)	0.4	0.4	0.7								
Dietary fiber (g)	4.5	5.8	2.6					**Potassium (K)/(mg)**	201	239	345
Total Sugar (g)	16.3	13.1						**Zinc (Zn)/(mg)**		0.5	6.7
Fructose (g)	2.2	1.7	2.6					**Copper (Cu)/(mg)**			22.2
Sucrose (g)	11.4	9.3	8.2					**Sodium (Na)/(mg)**	1.0		1.0
Glucose (g)	2.7	2.1	2.9								

Source: [1] Brannan et al., 2021; [2] Nam et al., 2018; [3] Galli et al., 2007; [4] Peterson, 1991; [5] USDA, 2005

PROCESSING

Pawpaw fruit can be eaten raw, processed and frozen. Ripe pawpaw fruit soften and have a powerful aroma (McGrath & Karahadian, 1994a; Shiota, 1991). The flavor of a pawpaw fruit can intensify as it over-ripens, as with banana, resulting in pulp that is excellent for use in cooking (Templeton et al., 2003).

Currently, pawpaws are primarily consumed as fresh fruit. Pawpaw fruit are best eaten fresh when fully ripe.

The intense tropical flavor and aroma (Shiota, 1991) also may be useful for developing processed food products (blended fruit drinks, baby food or puree, ice creams, juices, jam and other products, as are their *Annona* relatives.

Pawpaws easily substitute in equal part for banana in most recipes.

The flesh purees easily and freezes nicely (Alkofahi et al., 1989; Brannan & Wang, 2017; Jones & Layne, 1996; Rupprecht et al., 1986, 1990; Nam et al., 2018; Pomper & Layne, 2005).

Research suggests that pawpaw fruit pulp has the potential to be added to various consumer goods to add increased nutritional benefits or flavor enhancement. The intense, tropical-fruit-like flavor makes it a potential source of natural fruit flavor (Brannan et al., 2012; McGrath & Karahadian, 1994a).

Refrigeration of ripe pawpaw pulp exhibits no effect on phenolics, flavonoids, reducing potential, and radical scavenging compared to fresh pulp (Brannan & Wang, 2017; Harris & Brannan, 2009). Frozen pawpaw pulp is commercially available and usually includes ascorbic acid as a browning inhibitor. Nonetheless, frozen tissue browns very easily upon thawing and longer-term frozen storage (Brannan & Wang, 2017).

Enzymatic browning could have a significant effect on both food quality and food nutrition value. Enzymatic browning in pawpaw pulp produces a color deemed undesirable. Although commercial frozen pawpaw pulp preserved with ascorbic acid is on the market, anecdotal evidence suggests that this pulp browns during storage and especially quickly once thawed. A strategy to inhibit enzymatic browning during frozen storage would be useful for the nascent pawpaw industry (Brannan & Wang, 2017).

In the "The edible pawpaw – A collection of delicious and nutritious recipes" book, by the Ohio pawpaw growers association we found a lot of recipes with pawpaw fruits: breads, muffins and biscuits; cake; cream and cheesecake; cookies; custards; dips; ice cream and sherbet; pasta; pies; preserves; jam; puddings; dressing and sauce for salads; smoothie.

Pawpaws varies in flavor and intensity. The sugars in pawpaws are converted at high temperatures into caramel or butterscotch flavours, so the browner the cookies, the more these flavours develop at the expense of the pawpaw flavor. In the Figure 2. are presented most of all the possibilities for storing and processing pawpaw fruits.

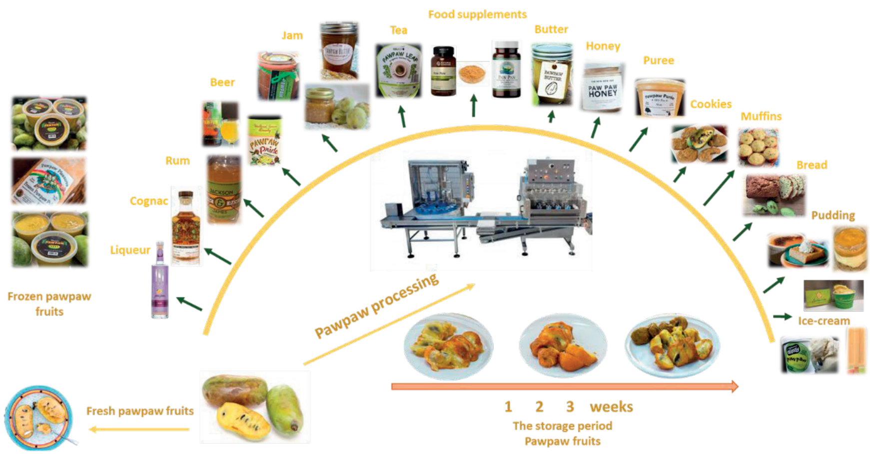

Figure 2. Processing of pawpaw - *Source: Original*

CONCLUSIONS

Asimina triloba (L.) Dunal is an interesting fruit and with a hight nutritional potential for the whole country, but also for Romania.

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INFLUENCE OF CLIMATIC FACTORS ON THE PHENOLOGICAL AND REPRODUCTIVE MANIFESTATIONS OF INTRODUCED APPLE CULTIVARS IN THE REGION OF TROYAN, BULGARY

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Abstract

The main phenological and biological features of the introduced apple cultivars were studied, for the period 2019-2021 in the region of Troyan. In order to establish their influence on the phenological manifestations of apple cultivars in order to be able to recommend them for cultivation from a practical point of view. It was found that in 2021 flowering was abundant and occurred 5-8 days later than the previous year. The latest date for the end of flowering was registered for the "Reanda" cultivar on May 12. The period from the beginning of flowering to its end is about 15-17 days. The fruits of the observed apple cultivars ripened in the period from mid-September to the first 10 days of October. The fruits of 'Remo' and 'Revena' reached the earliest stage of ripening, and 'Granny Smith' was the latest.

Key words*: Troyan region, climatic conditions, apple, cultivar, phenology.*

INTRODUCTION

The main ecological force of the phenology of fruit species in the temperate climate zone is temperature. Temperature increases because of global warming clearly have an impact. Nevertheless, predicting the effects of temperature fluctuations on tree phenology is challenging because the relationship between specific dormancy temperatures and blossoming time remains unclear. After a period of low temperatures, in order to overcome winter dormancy, buds require warm weather, which will trigger their growth (Fadón et al., 2021).

Kalvane et al. (2021) found that the blossoming and ripening period in apples are genetically determined to occur almost simultaneously: in the last week of May, in an interval of 4-5 days.

Koutinas (2010) presented new information on apple and cherry trees over the last 20-30 years, on factors and conditions that affect the flower bud formation and reproductive organ quality, paying attention to agronomics, particularly summer pruning, fertilizing, irrigation and treatment with growth regulators, through which the processes of flower bud formation can be regulated.

As a consequence of rising spring temperatures, apple blossoming in Europe has occurred significantly earlier over the last thirty to forty years with an average advance of 2-3 days per decade (Unterberger et al., 2018; Vitasse et al., 2018). Grauslund (1996) determined the blossoming dates of apple cultivars over a ten-year period (1985-94) and found that the beginning of flowering occurred about 1 month apart over the period, and the phenophase length varied from 16 to 37 days.

The long-term selection program of Dresden-Pilnitz apples are recognized and established cultivars resistant to economically important diseases and to critical changes in the conditions of climatic factors. Characteristics determining the influence of abiotic factors, for different ripening periods and the direction of use of the fruit production of these cultivars are combined (Fischer & Fischer, 2002).

To expand the assortment of apple cultivars for the Troyan region, trees of scab-resistant cultivars of the Re series and low susceptibility to powdery mildew of the Pi series were imported from Germany 20 years ago, which were selected in_the Institute in Dresden-Pillnitz (Peil et al., 2004).

At this stage of their cultivation in the conditions of the Troyan region have been established their growth and reproductive manifestations, biological and economic

significance, suitability for cultivation in the conditions of the region and the actual resistance to diseases (Dinkova et al., 2009; Stefanova et al., 2016). Correct identification of phenological stages in plants is very important for cultivar characterization, crop management, as well as disease and enemy management Martínez et al. (2019).
The analyzes on the phenology of development and the phenophases during the vegetation are the main key element for establishing the suitability of a given cultivar to the local agroecological conditions, in particular the climatic ones, such as temperature, precipitation, air humidity. In combination with the applied agricultural techniques, stable yields and quality of the fruit production can be guaranteed. Given the need for constant changes in the structure of the apple assortment and the fact that climate conditions have a significant impact on the yield and quality of fruits, the objective of the present study is to determine and describe the agroecological conditions of the Troyan region and their impact on the phenological performances of some apple cultivars, so that they can be recommended for cultivation from a practical point of view.

MATERIALS AND METHODS

Materials

The main phenological and biological features of the introduced apple cultivars were studied: 'Melrose', 'Reanda', Revena, Retina, Pilot, Topaz, Jonathan and Granny Smith, grafted on the rootstock MM 106, whereas Pingo, Remo, Reglindis, Idared, Grafenstein and Roseneisenapfel were on a seedling rootstock. The planting scheme is 5x4m. They are cultivated according to the generally accepted technology in RIMSA in Troyan, under nonirrigated conditions, without plant protection. The row-spacing are covered by grass.

Methods

The phenophases of flowering and fruit ripening were studied, according to the generally accepted methodology for studying plant resources in fruit plants (Nedev et al., 1979). The plot was periodically visited to observe each of the identified stages.

Experimental site

Climate factors for the study period (2019-2021) were compared with a 20-year base period. The factors were measured in a weather station on the territory of RIMSA Troyan. The climate is moderately continental with mild and warm winters and dry, not hot summers. The altitude is 420 m.

Indicators

Phenology   flowering and ripening periods of cultivars
Reproductive - yield per a tree (kg)

RESULTS AND DISCUSSIONS

Temperatures (°C) in March was higher by over 1°C in 2019 and 2020 than the base (6.1°C), and by 2.5°C lower in 2021 (3.6°C). In April, the trend is reversed as in 2019 the average monthly temperature was higher (10.2°C), whereas in 2021 and 2020 it was 1.3°C less than the base (10.7°C). The base average temperature in May was 15.3°C, as in 2021 it was the same, whereas in 2020 and 2019 it was 14.7°C, which was 0.6°C less (Table 1). Precipitation in the spring months of March, April, May was less than the base period (norm), except for April 2019, when it was 109 mm. The months of blossoming (end of March, April) had a lower average monthly temperature in 2021, compared to the previous 2 years. This strongly affects the blossoming periods of the studied apple cultivars by delaying it. The best precipitation amount was reported for the fruit ripening processes in 2020 in every summer month, incl. July, August and September, but the quantities were significantly smaller compared to the base 20 year period. The temperature values are the same as the base period, as in 2019 the same phenomena were reported, whereas the summer months in 2021 were with 2°C warmer than the average and there were extremely low amounts of precipitation (respectively 12; 56; 12 mm for July, August and September), and already in June the rainfall was only 64 mm, compared to 120 mm for the base period (Table 1).

Table 1. Climate factors (2019-2021) and average 20-year base period (2001-2020)

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2001-2020 precipitation (mm)	45.2	46.0	62.6	69.3	106.8	120.4	109.1	67.2	79.7	70.9	33.1	51.0
2021	82.8	25.6	47.7	57.0	82.8	64.8	12.4	56.2	11.8	72.8	14.4	68.6
2020	15.4	66.2	53.4	24.4	63.8	129.0	75.4	56.4	33.6	114.2	20.4	27.4
2019	40.4	55.2	16.5	106.9	82.4	234.6	106.7	37.7	21.9	16.2	29.6	16.8
2001-2020 T (°C)	-0.3	2.2	6.1	10.7	15.3	18.7	20.7	20.9	15.9	11.0	6.4	2.1
2021 T°C	1.6	3.7	3.6	9.4	15.4	18.9	22.7	22.7	16.2	8.7	7.5	2.6
2020 T°C	0.4	4.4	7.1	9.4	14.7	17.8	20.4	21.1	17.8	12.7	5.2	3.8
2019	-0.4	2.5	7.5	10.2	14.8	19.9	20.2	20.4	16.2	13.5	8.5	3.2

For the conditions of the warmer spring in 2019, the earliest start of blossoming period was registered in ‘Grafenstein’ cv. (March 29), followed by ‘Remo’ and ‘Reglindis’ (April 1), as ‘Melrose’ cv. registered the latest star of blossoming (11.04) (Figure 1). The same year was registered the longest flowering blossoming from 16 days for ‘Ravena’ to 25 days for ‘Melrose’ and ‘Pingo’. Throughout the month of April 2019, the average daily temperatures were from 8.5°C to 12.2°C with moderate rainfall, allowing the maintenance of favorable conditions for prolonged blossoming.
In 2020, blossoming was weak. It started on 15.04 for ‘Reglindis’, as this date was preceded by 5 consecutive days with temperatures above 10°C. Several days with low temperatures followed, and by 25.04 the lastest blossoming began for ‘Melrose’ and ‘Remo’ cultivars were the cultivars, which started their blossoming.
For the conditions of 2021, blossoming was abundant and took place 5-8 days later than the previous year. It started on 14.04 for ‘Grafenstein’, the last blossoming started for ‘Melrose’ 29.04, ‘Granny Smith’ and ‘Revena’ 28.04 (Figure 1). The latest date for the end of blossoming was registered for ‘Reanda’ cultivar on 12.05. Similar occurrences and timing were reported in 2015 by Stefanova et al. (2016) for the same cultivars. Kalvane et al. (2021) has been found that in recent decades the full blossoming of apple trees is earlier, i.e. for the sub-period (1959-1967) full blossoming occurred on May 28 (148th day of the year), and in 2002–2019 even earlier on May 21 (142 th day of the year).
From one year to the next, the order in which budding occurred is preserved, but the period of development changes depending on environmental factors, location, habitat, etc. (Cosmulescu et al., 2022). Gheorghiu & Cosmulescu (2022), determine that in depending on climatic conditions, the duration between phenophases differs by year, from the flowering stages of BBCH 60 "first open flowers" to BBCH 69 "end of flowering: all fallen petals") have a different duration on average 10 days in 2018 and 2020, 6.7 days in 2019 and 8.3 days in 2021.
For our study the total matching of all cultivars for each year is 6 days, as in 2019 it is in the period from April 06 to April 12, in 2020 from 24.04 to 30.04), in 2021 from 27.04 to 05.05. This is important for their mutual pollination and fertilizing.
‘Grafenstein’ apple cultivar is distinguished not only as an early bloomer but also as the earliest ripe. Its characteristic is that the fruits do not ripen at the same time. ‘Retina’, ‘Reglindis’ and ‘Remo’ reached ripening stage at the beginning of September, in 2019 even at the end of August. ‘Melrose’, ‘Pilot’ and ‘Revena’ trees ripened at the end of September, in 2021 even at the beginning of October (Figure 2). The harvesting period of individual cultivars is about 5-6 days, after which the fruits remain on the tree for a long time.

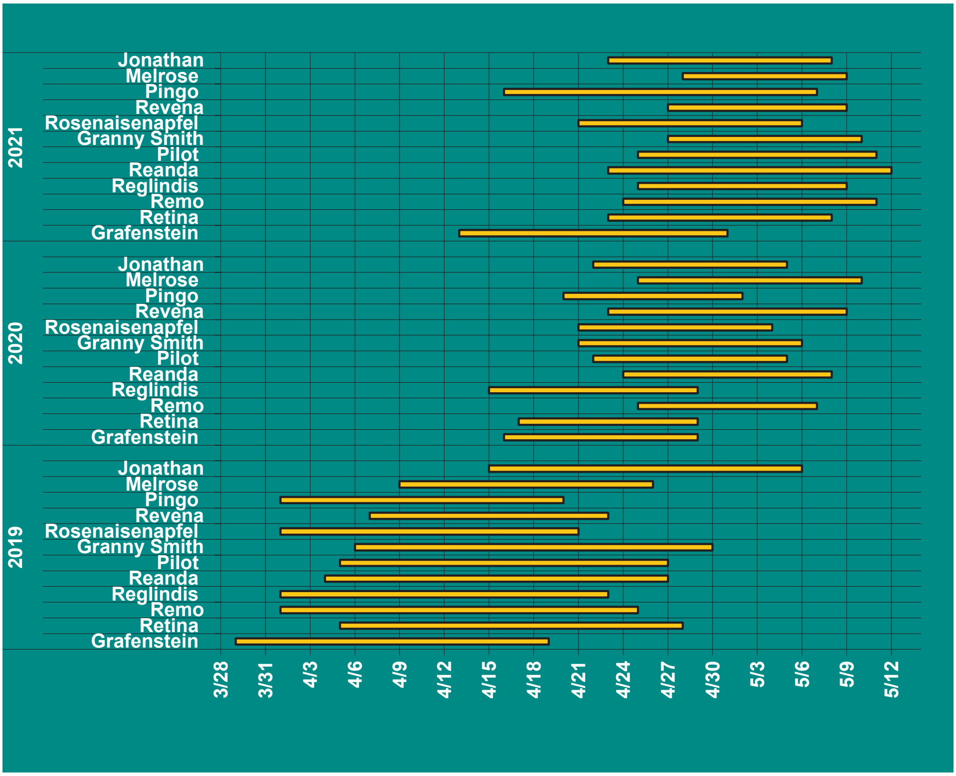

Figure 1. Blossoming phenogram (2019-2021)

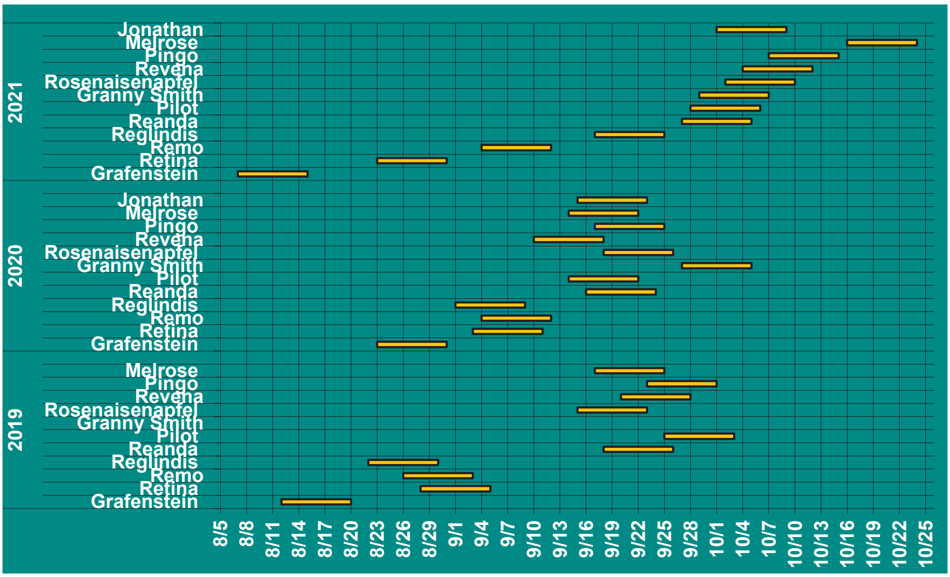

Figure 2. Ripening phenogram (2019-2021)

According to the period of fruit ripening, 'Reglindis' and 'Retina' are autumn cultivars, and Rewena and Topaz are winter. The highest 3-year total yield was obtained from 'Florina'

(75.1 kg/tree) and 'Reglindis' (63.6 kg/tree) trees Dimitrova & Sotirov (2020).
Favorable climatic conditions in 2019 allowed prolonged flowering and good pollination of flowers, retention of a useful knot and formation of the highest yields for the period of the study. The 'Grafenstein', 'Roseneisenapfel' and 'Reglindis' cultivars each having 80-90 kg/tree (Table 2). 'Retina' cultivar has the lowest yields 15-35 kg/tree, followed by 'Pilot' and 'Remo'.
As a result of the weak flowering in 2020, the apple cultivars produced poorly. In the earlier cultivars ('Retina', 'Reglindis', 'Grafenstein') there was no fruiting. The fruits that reached harvesting maturity were mainly from representatives of the winter cultivars, for which during ripening (September, October) sufficient humidity was not provided, precipitation was 35 mm (Table 1). In 2021, climate conditions favoured normal blossoming and the fruit bearing of apple cultivars was abundant. The highest yield for the 'Grafenstein' cv. was recorded (70.0 kg), and the lowest for 'Granny Smith' (7.0 kg). For the rest of the apple cultivars, the yield was relatively high (Table 2). Fruits that reached ripening stage were smaller in size than the average standard fruit for the cultivars studied. The fruits of the early ripening apple cultivars do not ripen together and remain on the tree for almost a month.
'Melrose', 'Pilot', 'Revena' (the latest ripening of the studied group) - ripen in early October (Figure 2), are larger, with a fruit weight > 100 g, and exhibit high drought resistance and the fruits manage to grow (plump) sufficiently.

Table 2. Reproductive parameters of apple cultivars (2019 and 2021)

	Fruit weight (g)		Yield (kg/tree)	
	2019	**2021**	**2019**	**2021**
Remo	112	93±15.10	50	45
Reglindis	100	80±11.22	80	40
Retina	126	69±5.41	35	15
Reanda	133	64±5.77	40	52
Revena	107	114±11.55	55	28
Rosenaisennapfel	150	104±13.04	85	15
Grafenstein	**106**	69±11.81	90	70
Pingo	134	128±15.18	70	55
Pilot	142	110±11.07	50	20
Melrose	156	83±4.28	65	41
Granny Smith	150	106±7.75	50	7
LSD 0,05		*13.66*		
LSD 0,01		*18.22*		
LSD 0,001		*23.81*		

Dinkova et al. (2009) registered the highest yields in 2006, for the initial fruit bearing period 2003-2007, as 'Reanda' and 'Revena' each having 28-30 kg yield per tree. In the present study, after 12 years of cultivation, the yields were significantly higher for these cultivars.
The extent to which plants are affected by changes in temperature and precipitation, their inherent capacity to adapt, will ultimately determine the potential for sustainable ecological stability and food security (Fitchett et al., 2015).

CONCLUSIONS

The studied apple cultivars from Re and Pi groups are suitable for cultivation in the mountaine region of Troyan and are recommended for enriching the assortment.
For each year of the study period, 'Grafenstein' was the earliest to blossom and 'Melrose' was the latest. 'Grafenstein' apple cultivar is distinguished not only as an early bloomer but also as the earliest ripener.
The cultivars 'Retina', 'Reglindis' and 'Remo' reach harvesting maturity at the beginning of September, 'Melrose', 'Pilot', 'Revena' at the

October. The largest are the fruits of the 'Pingo' and 'Pilot' cultivars.

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PRELIMINARY RESEARCH ON *IN VITRO* PROPAGATION OF *ZIZIPHUS JUJUBA* MILL.

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Abstract

In the present study, shoots of Ziziphus jujuba Mill. cv. 'Huping Zao' were used to identify an in vitro propagation protocol. Murashige and Skoog medium was used for this purpose. In the initiation and establishment stage, the best variant in terms of explant growth proved to be the one to which 2 mg L^{-1} IBA + 0.1 mg L^{-1} GA_3 + 1.5 mg L^{-1} NAA were added. Regarding the explant multiplication stage, the best results were obtained using 2 mg L^{-1} IBA, respectively 2 mg L^{-1} IBA + 0.5 mg L^{-1} NAA. The obtained results confirm the role and importance of different concentrations of hormones on the growth and development of explants.

Key words*: in vitro, jujube, tissue culture, shoots.*

INTRODUCTION

Ziziphus jujuba Mill. (jujube, Chinese date) belongs to the *Rhamnaceae* family, it is a fruit tree species originating in China for more than 7000 years and the place where most varieties are found (Liu et al., 2020; Jin, 2018). Jujube is intensively cultivated mainly in China, USA, India, Middle East, Australia, Italy (Liu et al., 2020) due to its beneficial properties on health (Stan et al., 2021; Chen et al., 2019; Cosmulescu et al., 2018), used in traditional medicine as anti-inflammatory, detoxifying, antioxidant, antimicrobial, gastrointestinal protective, cardiovascular, anticancer (Liu et al., 2021). It is a species that is quite difficult to multiply by generative methods (Karimpour et al., 2013; Sapkota et al., 2020; Rahaman et al., 2018) and if this is possible, the percentage of germination is very low (Stănică, 2019). The vegetative propagation methods among the most used are grafting, propagation by cuttings and shoots (Stănică, 2019; Yao, 2016), but if the procedure is not carried out correctly, the chances of success are non-existent and also the lack of rootstocks makes this aspect very difficult. A rapid propagation method is tissue culture (buds, shoots, leaves, callus) or *in vitro* micropropagation (Dai et al., 2009; Gu & Zhang, 2005; Khazaei et al., 2015). According to Hussain et al. (2012) plant tissue culture allows the growth of whole plants, organs, tissues or cells under controlled aseptic conditions in the laboratory, provides all the nutrients, energy and water necessary for explant growth through the used culture medium, and the explants development can then be manipulated by adding growth hormones (Phillips & Garda, 2019) depending on the pursued objectives (callusogenesis, organogenesis, rhizogenesis). This method has advantages and disadvantages. Among the advantages we can mention the rapid micropropagation (Iliev et al., 2010), in a relatively short time, of a large number of plants identical to the donor plant (the mother plant from which the plant material is harvested), obtaining clones, viruse free plants, grown under aseptic conditions and controlled environmental factors (George et al., 2008). The process is laborious, expensive (Liu et al., 2015), it is carried out in sterile laboratory conditions, and special attention must be paid to the preparation of the plant material in order to have a chance of success (Rahaman et al., 2018). Of course, the most common culture medium used by researchers for *in vitro* propagation of jujube is the Murashige and Skoog culture medium (Yıldırım et al., 2015; San et al., 2014; Zhou & Liu, 2009; Goyal et al., 2006; Jian et al., 2006; Gu & Zhang, 2005) supplemented with different growth hormones in different concentrations. Due to climate changes, i.e. increasing temperatures, lengthening of the growing seasons, increasing periods of drought on the territory of Romania, as well as the strategic position on the globe, the Chinese jujube can be considered a suitable species for future orchards or as individual plants in private gardens (Stănică, 2019). Recent studies have demonstrated the adaptability of this species to the climate of our country, in teaching nurseries, research stations, private gardens, but especially due to the existence of the spontaneous jujube ("Dobrogea olive") present in Dobrogea area, Southeast part of Romania (Stănică, 2019). The purpose of this research paper was to establish a proper sterilization protocol for jujube explants, i.e. to find an appropriate growth medium and hormonal balance for their successful micropropagation.

MATERIALS AND METHODS

Plant material and sterilisation

The biological material was represented by *Ziziphus jujuba* Mill. cv. 'Huping Zao'. Jujube branches were harvested from the collection orchard of USAMV of Bucharest during dormancy (BBCH 00), transported to the laboratory and subjected to a fungicidal treatment by surface sterilization using 0.1% Thiophanate methyl 70% and 0.2% Mancozeb 80%. The branches were kept in water at room temperature (24 ± 2°C) until the time of onset of vegetation (BBCH 10-11/31), when the shoots were detached and subjected to two methods of sterilization under aseptic conditions. The first method was the one described by Soliman and Hegazi (2013), namely washing the explants with detergent for 5 minutes, then rinsing them with an antioxidant solution (1 g of ascorbic acid + 5 g of citric acid dissolved in 200 mL of sterile distilled water), followed by washing for 15 minutes with a 20% sodium hypochlorite solution, then one minute with a disinfectant solution (2 mL disinfectant + 200 mL sterile distilled water) and finally 6 rinses with sterile distilled water. The second sterilization method consisted of washing the explants for 20 minutes with water and detergent, followed by 20 minutes with 40% sodium hypochlorite solution, a rinse with alcohol (70%) for 10 seconds, respectively 6 rinses with sterile distilled water.

Culture medium and conditions

Explants were grown on MS culture medium (M5519, Sigma-Aldrich) supplemented with 3% sucrose (w/v) and gelled with 7 g L^{-1} agar. Different concentrations of growth hormones (Sigma-Aldrich) on 4 variants in the case of the initiation and establishment stage, respectively 3 variants in the case of the multiplication and transfer stage were used (Table 1). The pH of the initial medium was 4.6 after preparation, and adjusted to 5.8 before autoclaving (Raypa AES-8 for 20 minutes at 121°C) by adding 1M NaOH. After sterilization, the culture medium was distributed in sterile Erlenmeyer flasks in a laminar flow hood. After inoculation, the explants were incubated in a climatic chamber (Sanyo MLR351) at a temperature of 24 ± 2°C, 3000 lux light intensity and 70% humidity, with a photoperiod of 16 hours. After 40 days on initiation medium, explants were multiplied and transferred to MS culture medium using 3 variants of hormones (Table 1). The explants were carefully cut, brown spots and yellowed leaves were carefully removed, so that only perfectly healthy explants were inoculated on the new culture medium.

Table 1. The used growth regulators

Stage	Variant	Growth hormones (mg L^{-1})*				
		TDZ	BAP	IBA	GA_3	NAA
Initiation and establishment	A_1	0.25		0.1		
	A_2		0.5	0.1		
	A_3			1.5	0.1	
	A_4			2.0	0.1	1.5
Multiplication and transfer	V_1			2.0		
	V_2			2.0		0.5
	V_3			1.0		

*TDZ-thidiazuron; BAP–benzyl amino purine; IBA-indole-3-butyric acid; GA3-Gibberellic acid; NAA-1-Naphthylacetic acid

Statistical analysis

Measurements of shoot height (starting from the surface of the culture medium), shoot diameter (expansion at the widest points taking into account the tips of the leaves) and number of leaves per shoot were made. The obtained data were processed in the IBM SPSS Statistics 26 program and represent the mean, standard deviation, the limits of variation and the coefficient of variability.

RESULTS AND DISCUSSIONS

Important in the case of disinfection of plant material from the natural environment, consists in the application of an appropriate fungicidal treatment. Hansika et al. (2017) used Captan 50%, 1.2 g L^{-1}, Thiophanate methyl 70%, 2 g L^{-1} and Chlorothalonil, 1.8 mL L^{-1} as a disinfectant for jujube explants, the best results being obtained by immersing shoots in Captan for 20 minutes with a 79.9% success rate. The treatment applied by surface sterilization in this study (0.1% Thiophanate methyl 70% and 0.2% Mancozeb 80%) had a success rate of 87.3%. Regarding the sterilization of explants, both used variants gave promising results with their survival percentage of 77% in the case of the first sterilization variant, while Soliman and Hegazi (2013) had an explant survival rate of 86% using the same protocol, respectively 92% in the case of sterilization option number two. Safarnejad (2015) mentions in his study the sterilization of jujube buds using 0.02% $HgCl_2$ for 3 minutes, 70% ethanol for 2 minutes and 30% NaOCl for 15 minutes. Also Yıldırım et al. (2015) sterilized jujube shoots by immersion in 3% NaOCl (v/v) for 18 minutes, followed by three rinses in sterile distilled water for 5 minutes. Another sterilization option with a yield of 65.5% is that described by Melyan et al. (2014) using 2% $Ca(ClO)_2$ for 15 min and 70% ethanol for 3 min. Khazaei et al. (2015) mention the use of 70% ethanol for 1 minute and 2% sodium hypoclorite for 25 minutes, followed by rinsing with distilled water for 25 minutes for jujube buds disinfection protocol.

The best variant in the initiation and establishment stage of the *in vitro* culture was the A_4 variant in terms of all three determined morphological characteristics, namely the height of the explants (with an average of 1.77 cm), the diameter of the shoots (with a value average of 1.58 cm) and the number of leaves (with an average of 5.67 leaves/shoot) (Table 2). This demonstrates the role of growth hormones used 2 mg L^{-1} IBA + 0.1 mg L^{-1} GA3 + 1.5 mg L^{-1} NAA compared to the other variants. Yildirim et al. (2015) had the best results using the combination of 0.1 mg L^{-1} TDZ + 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} IBA + 0.3 mg L^{-1} GA_3 in the production of new shoots in *Ziziphus jujuba* multiplied *in vitro*. At the same time, Ma et al. (2012) confirmed the use of thidiazuron (along with $AgNO_3$ and NAA) as beneficial in the shoot regeneration process and Wang et al. (2013) the use of thidiazuron in combination with IBA in the process of new shoot emergence. Huo et al. (2007) mention IBA in a concentration of 0.2 mg L^{-1} along with 5 mg L^{-1} BA as being beneficial for the proliferation of jujube explants in the variety 'Gagazao'. The high coefficient of variability indicates large differences between the inoculated Erlenmeyer flasks within each variant. Herman (2015) mentions IBA and NAA as being among the most used auxins in tissue culture for cell division, callus formation, shoot growth and rooting. Thidiazuron is considered a frequently used cytokinin in the case of woody species (Huetteman & Preece, 1993) but with a lower incidence compared to BAP. Gibberellic acid (GA_3) was successfully used for jujube shoot elongation along with benzylaminopurine by Melyan et al. (2014).

Table 2. Results obtained during the initiation and establishment phase

Var	Descriptive statistics*	Shoot height (cm)	Shoot diameter (cm)	Number of leaves
A_1	Mean±SD	1.24 ± 0.63	1.48 ± 0.93	4.21 ± 1.55
	Range	0.30-3.00	0.10-3.80	1.00-7.00
	CV%	50.80	62.83	36.81
A_2	Mean±SD	1.28 ± 0.45	1.54 ± 0.97	3.83 ± 2.41
	Range	0.40-2.10	0.20-3.60	0.00-1.00
	CV%	35.15	59.74	62.92
A_3	Mean±SD	1.30 ± 0.76	1.15 ± 0.66	4.29 ± 1.81
	Range	0.30-2.70	0.50-2.30	1.00-7.00
	CV%	58.46	57.39	42.19
A_4	Mean±SD	1.77 ± 0.23	1.58 ± 0.72	5.67 ± 0.52
	Range	1.40-2.00	0.80-2.40	5.00-6.00
	CV%	12.99	45.56	9.17

*SD = Standard Deviation; CV% = Coefficient of variation

Hao et al. (2013) successfully used a hormone balance composed of thidiazuron and NAA in MS medium for callus culture and subsequent shoot emergence of jujube. The hormonal variant with thidiazuron in this study had the lowest values in terms of shoot elongation

(with an average of 1.24 cm) followed by the variant with BAP (with an average of 1.28 cm) in terms of the same characteristic using 0.25 mg L^{-1}. Zhou and Liu (2009) confirm the use of gibberellic acid as having an important role in the elongation of the shoots, respectively 0.5 mg L^{-1} in the case of 'Dongzao' shoots, results consistent with those obtained in this work, the two variants with GA_3 having the higher results regarding this characteristic (but in a lower concentration 0.1 mg L^{-1}).
Regarding the multiplication and transfer stage, although no root emergence was observed, the best effect on shoot height growth and leaf emergence and formation was 2 mg L^{-1} IBA, with mean values of 2.01 cm, respectively 5.30 leaves/shoot. V_2 was beneficial to shoot diameter expansion, respectively 2 mg L^{-1} IBA + 0.5 mg L^{-1} NAA, with an average of 1.71 cm. Yıldırım et al. (2015) suggest the use of 2 mg L^{-1} IBA as beneficial to root formation (with a percentage of 76.7% root formation in their study) (Table 3). In the present study the same concentration of IBA used had no impact on rhizogenesis formation. The high coefficient of variation indicates a high degree of variability within the determinations performed and between variants.

Table 3. The results obtained in the multiplication and transfer stage

Var	Descriptive statistics*	Shoot height (cm)	Shoot diameter (cm)	Number of leaves
V_1	Mean±SD	2.01±0.31	1.51±0.42	5.30±0.73
	Range	1.10-2.60	0.80-2.50	4-7
	CV%	15.42	27.81	13.77
V_2	Mean±SD	1.91 ± 0.37	1.71 ± 0.62	5.00±1.64
	Range	1.40-2.50	1.00-3.30	3-8
	CV%	19.37	36.25	32.80
V_3	Mean±SD	1.30 ± 0.55	0.64 ± 0.47	2.00±2.74
	Range	0.80-1.90	0.30-1.20	0-5
	CV%	42.30	73.43	137

*SD = Standard Deviation; CV% = Coefficient of variation

Hansika et al. (2017) used MS culture medium supplemented with 1.5 mg L^{-1} BAP and obtained a percentage of 96.66% in terms of shoot elongation, and the lowest values using TDZ 0.2 mg L^{-1} (3.33%), but with callus production. They also mention the lack of formation of new shoots in any of the two variants used and the lack of root formation on medium supplemented with IBA, results consistent with those obtained in this paper. Future research on other hormone combinations will be conducted to identify the best variant in terms of emergence and formation of new shoots and root formation respectively. The obtained results confirm the role and influence of hormones on the growth and development of jujube explants. Compliance with the work protocol, starting from the choice of explants, their disinfection, sterilization, inoculation and ensuring the environmental factors necessary for development, is the key to the success of the culture initiation and establishment stage. Aseptic conditions are the most important aspect of tissue cultures to avoid unwanted infections. The culture medium and the hormonal balance are the decisive factors in the appearance and development of certain morphological processes (differentiation, dedifferentiation, callus formation, shoot elongation, leaf growth, new shoot formation).

CONCLUSIONS

Growth hormones influence the growth and development processes of explants. MS culture medium using the combination of IBA, GA_3 and NAA was found to be beneficial during the initiation and establishment stage of the culture. Special attention must also be paid to the sterilization of the explants in order to have as high a percentage of success as possible. None of the hormonal variants caused the emergence of new shoots and the formation of roots. Further research on increasing the concentration of hormones but also the use of others will be carried out to establish a suitable protocol for the *in vitro* propagation of the Chinese jujube.

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PRELIMINARY RESULTS REGARDING YIELD AND FRUIT QUALITY OF SOME APPLE CULTIVARS IN ECOLOGICAL SYSTEM

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Abstract

The aim of this study was to assess the yield and fruits quality of apple produced in ecological system. In 2022 the influence of different fertilizers on yield and fruits quality at three apple cultivars was carried out. The trees were planted in a spacing of 3 x 2 m, according to the following experimental scheme: Factor A - cultivar, with three graduation (a1 - 'Romus 3', a2 - 'Idared' and a3 - 'Golden delicious'); Factor B - fertilization variant, with four graduations (b1 - Biohumus - 0.5 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; b2 - Biohumus - 0.7 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; b3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; b4 - 'Unfertilized'). As results of the investigations we found that the highest fruit yield and fruits quality was obtained at 'Idared' (28.66 kg/tree, respectively 215.66 g) cultivar in fertilization variant 3.

Key words*: apple, cultivar, fertilizers, yield, fruits quality.*

INTRODUCTION

In the last decade, consumers started to look to ecological products which have lower environmental impacts and higher nutritive values (Amarante et al., 2008, Cuevas et al., 2015; Butac & Chivu, 2020).
In ecological agriculture, the use of synthetic pesticides and fertilizers is not allowed, but only organic ones such as animal and green manure, compost, sulfur and copper products, pheromone traps and other biological control methods (Holb et al., 2003; Peck et al., 2006; Jonsson, 2007; Amarante et al., 2008; Butac et al., 2021).
Ecological apple production is still quite limited in most European countries, due to the reduced possibilities to control the diseases and pests (Jönsson, 2007; Amarante et al., 2008), as well as due to the lack of organic fertilizers (McArtney & Walker, 2004), which limits profitability of ecological apple orchards.In Romania, ecological agriculture included, at the level of 2019, an area of approximately 395,228 ha, respectively 2.9% of the agricultural area. Fruit trees occupy only 15,905 ha, i.e. a share of 4.0% of the total organic agriculture at national level (Butac et al., 2021). The objective of this paper was to evaluate yield and some important quality parameters of three apple cultivars from orchards managed under ecological system in Maracineni - Arges area, Romania.

MATERIALS AND METHODS

The experience was carried out in a demonstrative plot established in 2010 at Maracineni, in a private farm from Arges county, Romania. The trees were planted in a spacing of 3 m between the rows and 2 m between trees, according to the experimental scheme from Table 1.

Table 1. Experimental scheme

Factor	Variant/Origin	Doses and method of application
A - Cultivar	V1. Romus 3 -Romania	-
	V2. Idared - USA	-
	V3. Golden delicious -USA	-
B - Feritilization variant	V1. Biohumus + Macys BC 28 + Cifamin BK	- 0.5 L/tree, soil - 2 L/ha, foliar - 1 L/ha, foliar
	V1. Biohumus + Macys BC 28 + Cifamin BK	- 0.7 L/tree, soil - 2 L/ha, foliar - 1 L/ha, foliar
	V1. Biohumus + Macys BC 28 + Cifamin BK	- 0.9 L/tree, soil - 2 L/ha, foliar - 1 L/ha, foliar
	V4.	Unfertilized

The determinations were carried out in 2022 year. Biohumus fertilizer was applied in spring before the start of vegetation and in autumn after the fall of the leaves. Macys BC 28 and Cifamin BK fertilizers were applied after flowering and in the young fruit phase. The experiment was carried out in a randomized block design, in 3 replicates with 3 trees per plot.

In these experimental variants we carried out the following parameters: fruits yield in kg/tree; fruits weight in g; fruits soluble solids content with a digital refractometer in ºBrix; pH of fruits with the device Minititrator Hanna Instrument 84532; fruits firmness was measured with non-destructive penetrometer Qualitest HPE.

The results of the experiment were analyzed statistically using Duncan‘s multiple range test at a 0.05% significance level.

RESULTS AND DISCUSSIONS

Fruits yield. Between the fertilized and unfertilized variants there are significant differences of fruits yield. The 'Idared' cv. registered the highest fruit production (24.83 kg/tree) significantly exceeding the 'Romus 3' (20.33 kg/tree). With the increase of Biohumus doses, the production of fruits also increases (from 23.11 kg/tree in V1 to 25.77 kg/tree in V3) (Table 2).

Table 2. Influence of the fertilizers on the yield (kg/tree)

Cultivar	Fertilization variant*				
	V1	V2	V3	V4	Average**
Romus 3	21.33	21.66	22.00	16.33	**20.33 b**
Idared	24.66	27.66	28.66	18.33	**24.83 a**
Golden delicious	23.33	24.33	26.66	18.66	**23.24 a**
Average**	**23.11 b**	**24.55 ab**	**25.77 a**	**17.77 c**	

*V1 - Biohumus - 0.5 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V2 - Biohumus - 0.7 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V4 - Unfertilized

**Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05).

The fertilization variant 3 determined a higher fruit production than the other variants, respectively 25.77 kg/tree, exceeding the fruits production obtained in V1 with 2.66 kg/tree, with 1.22 kg/tree in V2 and with 8.00 kg/tree in unfertilized variant. In conclusion, it can be said that among all the 3 varieties studied, the highest fruit production was obtained in the fertilization variant 3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application (Table 2).

Fruits weight. The 'Idared' cv. registered the highest fruit weight (215.16 g) significantly exceeding the 'Romus 3' (121.91 g) and 'Golden delicious' (185.33 g). With the increase of Biohumus doses, the fruits weight also increases (from 178.00 g in V1 to 213.55 g in V3).

The fertilization variant 3 determined a higher fruit weight than the other variants, respectively 213.55 g, exceeding the fruits weight from V1 with 35.55 g, with 42.99 g in V2 and with 79.22 g in unfertilized variant. In conclusion, it can be said that among all the 3 varieties studied, the highest fruit weight was obtained in the fertilization variant 3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application (Table 3).

Table 3. Influence of the fertilizers on the fruits weight (g)

Cultivar	Fertilization variant*				
	V1	V2	V3	V4	Average**
Romus 3	120.66	108.66	150.00	108.33	**121.91 c**
Idared	212.00	223,33	271.33	154.00	**215.16 a**
Golden delicious	201.33	180.00	219.33	140.66	**185.33 b**
Average**	**178.00 b**	**170.66 b**	**213.55 a**	**134.33 c**	

*V1 - Biohumus - 0.5 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V2 - Biohumus - 0.7 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V4 - Unfertilized

**Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05).

Fruits soluble solids content. On average of the experimental variants, the highest soluble solids content was recorded on the 'Golden delicious' cv. (12.75ºBrix), followed by the 'Idared' cv (12.19ºBrix).

The highest fruits soluble solids content was obtained in the fertilization variant 3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application (12.68º Brix) (Table 4).

Table 4. Influence of the fertilizers on the fruits soluble solids content (^{o} Brix)

Cultivar	Fertilization variant*				
	V1	V2	V3	V4	Average**
Romus 3	11.33	12.06	11.53	11.56	**11.62 b**
Idared	12.16	11.90	13.06	11.63	**12.19 ab**
Golden delicious	11.36	12.80	13.46	13,40	**12.75 a**
Average**	**11.62 c**	**12.25 b**	**12.68 a**	**12.20 b**	

*V1 - Biohumus - 0.5 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK – 1 L/ha, foliar application; V2 - Biohumus - 0.7 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V4 - Unfertilized
**Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05).

Fruits pH. On average of the experimental variants, the highest value of fruits pH was obtained on the 'Golden delicious' cv. (4.22), which indicates sweeter fruits compared to the 'Romus 3' (3.86) and 'Idared' (3.92) cultivars. The highest value of fruits pH was obtained in the unfertilized variant (4.02) and the lowest pH value of the fruits was obtained in variant 3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application (3.99) (Table 5).

Table 5. Influence of the fertilizers on the fruits pH

Cultivar	Fertilization variant				
	V1	V2	V3	V4	Average**
Romus 3	3.76	3.95	3.83	3.89	**3.86 b**
Idared	3.85	3.90	3.92	4.02	**3.92 b**
Golden delicious	4.38	4.15	4.23	4.14	**4.22 a**
Average**	**4.00 a**	**4.00 a**	**3.99 a**	**4.02 a**	

*V1 - Biohumus - 0.5 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V2 - Biohumus - 0.7 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V4 - Unfertilized
**Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05).

Fruits firmness. On average 'Idared' cv. had firmer fruits than 'Romus 3' and 'Golden delicious' cvs., between these cultivars being differences but not statistically assured (Table 6).
The fruits of all cultivars studied had higher flesh firmness at harvest time in all fertilization variants than unfertilized variant (Table 5), results confirmed by other authors as well DeEll and Prange (1992), Reganold et al. (2001), Weibel et al. (2004), Peck et al. (2006) at apple.

Table 6. Influence of the fertilizers on the fruits firmness (HPE units)

Cultivar	Fertilization variant*				
	V1	V2	V3	V4	Average**
Romus 3	68.63	75.66	69.23	69.06	**70.64 a**
Idared	73.60	71.10	71.13	70.23	**71.52 a**
Golden delicious	73.30	72.66	70.96	65.06	**70.50 a**
Average**	**71.84 a**	**73.14 a**	**70.44 b**	**68.12 c**	

*V1 - Biohumus - 0.5 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V2 - Biohumus - 0.7 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V3 - Biohumus - 0.9 L/tree, soil application + Macys BC 28 - 2 L/ha, foliar application + Cifamin BK - 1 L/ha, foliar application; V4 - Unfertilized
**Duncan multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05).

CONCLUSIONS

The culture of fruit trees in an ecological system has a positive influence both on the environment and on the fruits yield and their quality.
The results of our study showed that the production and fruits quality had higher values in the case of the fertilized variants compared to the unfertilized variant.
Also, with the increase of Biohumus doses, the production and fruits quality increases at all cultivars studied.

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CHEMICAL ANALYSIS OF SOME LOCAL AND CULTIVATED JUJUBE GENOTYPES (*ZIZIPHUS JUJUBA* MILL.) FROM DOBROGEA REGION

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Abstract

The cultivation of Chinese jujube can be traced back 7,000 years to the Neolithic era, making it one of the oldest cultivated fruit plants in the world. In Romania, Chinese jujube can be found in nature, semi-spontaneously, in Jurilovca and Mahmudia in Tulcea County and Ostrov in Constanța County. This paper describes the genotypes of Chinese jujube (Ziziphus jujuba Mill.) found in the Dobrogea region, specifically in Jurilovca, Mahmudia, Bugeac, and Ostrov. The study focuses on the chemical analysis and mineral composition of seven local jujube genotypes from Dobrogea and five Chinese cultivars. In the analyzed jujubes, sugar content varied between 15.91% to 31.07%; dry matter varied between 19.50% and 39.00%; total polyphenols varied between 390 to 1,020 mg gallic acid/100 g, calcium varied between 6.29 and 47.95 mg/100 g.

Key words*: Chinese jujube, polyphenols, sugar content, mineral composition.*

INTRODUCTION

Ziziphus jujuba Mill., also known as jujube in English or Zao in Chinese, originates from the middle and lower regions of the Yellow River, the "mother river" of the Chinese people. Its fruits are often confused with Indian jujube (*Ziziphus mauritiana*), but these are two different species.

The *Ziziphus jujuba* tree produces flowers in early summer (June-July), and the fruits ripen gradually in autumn (September-October). The fruits can be consumed fresh, dried, or processed into juices, wines, syrups, preserves, candies, and vinegar.

Jujube stands out with its unique composition, encompassing 23 types of amino acids rarely present in other fruits. This diversity contributes to its reputation as a traditional and functional Chinese food renowned for its numerous health benefits. The primary minerals in jujube include phosphorus, potassium, calcium, and manganese. Notably, sodium, zinc, copper, and iron are also present (Shahrajabian, 2019).

The cultivation of Chinese jujube can be traced back 7,000 years to the Neolithic era, making it one of the oldest cultivated fruit plants in the world. During the Han Dynasty, approximately 2,000 years ago, the cultivation of Chinese jujube spread throughout the entire region of Northern and Southern China (Liu, 2020).

In Romania, Chinese jujube can be found in nature, semi-spontaneously, in the localities of Jurilovca, Mahmudia in Tulcea County, and Ostrov in Constanța County. It is believed that they reached our country through the Silk Road and routes near ancient Greek, Roman, and Byzantine cities, such as the Argamum Fortress (Jurilovca, Tulcea County), Enisala Fortress (a fortress overlooking the Razim and Babadag lakes today), Salsovia Fortress (Mahmudia, Tulcea County), and Vicina Fortress (located on Păcuiul lui Soare Island, also known as the sunken fortress in the Ostrov area, Constanța County) (Stănică, 2019).

MATERIALS AND METHODS

The study was conducted by analyzing the fruits of seven wild jujube genotypes identified in Tulcea and Constanța County, namely the Jurilovca, Mahmudia, Bugeac, and Ostrov genotypes, and on five jujube cultivars, namely Taigu Ban, Hu Ping, Xuan Cheng Jiang Zao, Jun Zao, and Hongan.

The cultivars of Chinese jujube were analyzed to compare wild jujube genotypes for a better understanding.

This study aims to present several biochemical characteristics of the fruits, such as sugar content (%), soluble dry matter (%), acidity (citric acid%/100 g), total polyphenols (mg gallic acid/100 g), and antioxidant capacity (mg Trolox/100 g). Additionally, the mineral content, including lead (µg/kg), cadmium (µg/kg), chromium (µg/kg), copper (mg/kg), zinc (mg/kg), manganese (mg/kg), iron (mg/kg), sodium (mg/100 g), potassium (mg/100g), calcium (mg/100 g), and magnesium (mg/100 g), were also analyzed.

RESULTS AND DISCUSSIONS

The presented data shows that sugar levels varied significantly among the samples of Chinese jujube fruits, with values ranging from 15.91% to 31.07%.

The Jurilovca III 1, Ostrov, and Jurilovca I 1 genotype exhibit the highest sugar levels, 31.07%, 29.90%, and 26.40%, respectively, while the Hu Ping, Jun Zao, and Hongan varieties had the lowest sugar levels, 15.91%, 16.35%, and 17.45% (Table 1, Figure 1).

The presented data in Figure 2 refers to the acidity level expressed as citric acid (%) measured in different samples of Chinese jujube fruits. Acidity is an essential characteristic of fruits and can influence their taste and quality.

Acidity levels vary significantly among genotypes, ranging from 0.26% to 1.05%.

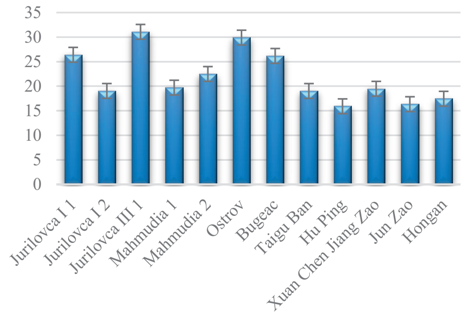

Figure 1. Sugar content (%)

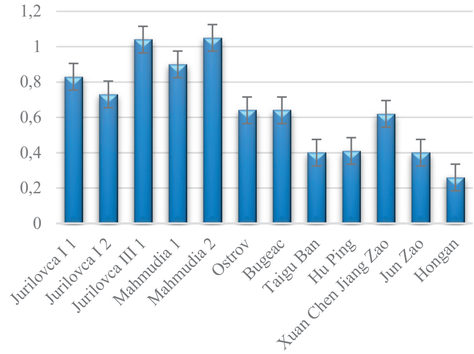

Figure 2. Acidity (citric acid %/100 g)

The Mahmudia 2, Jurilovca III 1, and Mahmudia 1 genotypes exhibit the highest acidity levels, 1.05%, 1.04%, and 0.90%. At the same time, the fruits of the Hongan, Taigu Ban, and Jun Zao varieties have the lowest acidity levels, 0.26%, 0.40%, and 0.40%, respectively (Table 1.)

Table 1. Biochemical analyses

Genotype	Sugar content (%)	Acidity (citric acid %/ 100 g)	Soluble dry matter (%)	Total polyphenols (mg gallic acid/ 100 g)	Antioxidant capacity (mg Trolox/100 g)
Jurilovca I 1	**26.4**	**0.83**	25.50	**690**	**375.0**
Jurilovca I 2	19.04	**0.73**	24.75	**750**	**382.5**
Jurilovca III 1	**31.07**	**1.04**	**27.00**	**780**	**405.0**
Mahmudia 1	19.71	**0.9**	21.75	**720**	**397.5**
Mahmudia 2	**22.49**	**1.05**	**30.00**	**1020**	**442.5**
Ostrov	**29.9**	0.64	**39.00**	510	232.5
Bugeac	**26.17**	0.64	**34.5**	**690**	**315.0**
Taigu Ban	19.03	0.40	21.00	420	142.5
Hu Ping	15.91	0.41	19.50	390	120.0
Xuan Chen Jiang Zao	19.48	0.62	19.50	540	270.0
Jun Zao	16.35	0.40	21.00	480	202.5
Hongan	17.45	0.26	24.00	450	240.0

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Dry matter content (%) measured in different fruit genotypes is presented in Figure 3, being an indicator of fruit quality.

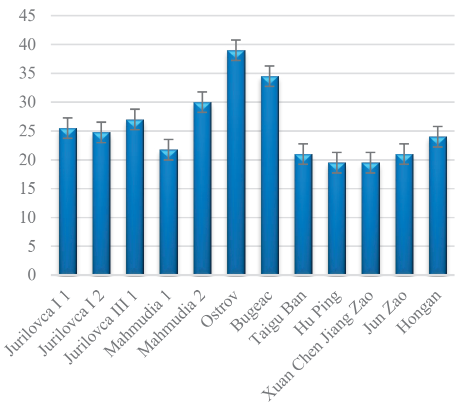

Figure 3. Dry matter (%)

The dry matter levels varied significantly among genotypes, ranging from 19.5% to 39%. The Ostrov, Bugeac, and Mahmudia 2 genotypes exhibit the highest levels of soluble dry matter, 39.00%, 34.50%, and 30.00%. At the same time, the fruits of the analyzed cultivars Hu Ping, Xuan Chen Jiang Zao, and Jun Zao had the lowest levels for dry matter, 19.50%, 19.50%, and 21.00%.

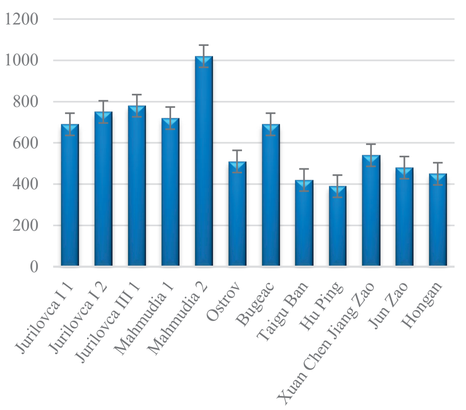

Figure 4. Total polyphenols (mg gallic acid/100 g)

Polyphenols are organic compounds found in plants and are considered beneficial for health as they can help prevent chronic diseases such as cardiovascular diseases and cancer. In general, foods that contain high levels of polyphenols are considered healthy, and consuming them can benefit health.

Total polyphenol levels varied significantly among samples of Chinese jujube fruits, ranging from 390 to 1,020 mg gallic acid/ 100 g. Mahmudia 2, Jurilovca III 1, and Jurilovca I 2 exhibit the highest levels of total polyphenols, with values of 1,020 mg gallic acid/100 g, 780 mg gallic acid/100 g, and 750 mg gallic acid/100 g, respectively. At the same time, the fruits of the analyzed cultivars Hu Ping, Taigu Ban, and Hongan had the lowest levels of total polyphenols, with values of 390 mg gallic acid/100 g, 420 mg gallic acid/100 g, and 450 mg gallic acid/100 g, respectively (Table 1., Figure 4).

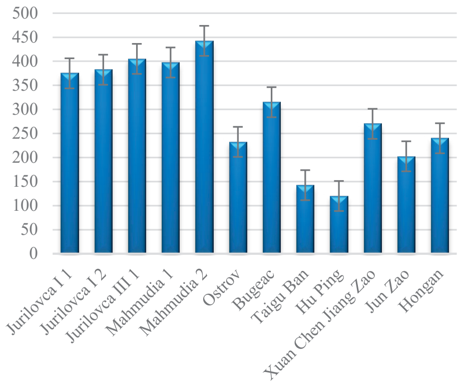

Figure 5. Antioxidant capacity (mg Trolox/100 g)

Antioxidant capacity refers to the ability of a substance to neutralize or prevent cellular damage caused by free radicals and other reactive oxygen species. Antioxidants can be found in various foods, including fruits and vegetables, and help protect cells and tissues from oxidative stress and the adverse effects of aging and chronic diseases.

According to the presented data, antioxidant capacity measured by the DPPH method varied significantly among genotypes, with values ranging from 120 to 442.5 mg Trolox/100 g. Mahmudia 2, Jurilovca III 1, and Mahmudia 1 genotypes exhibited the highest antioxidant capacities with values of 442.50 mg Trolox/ 100 g, 405 mg Trolox/100 g, and 397.50 mg Trolox/100 g, while the fruit samples of the Chinese jujube cultivars Hu Ping, Taigu Ban, and Jun Zao had the lowest antioxidant capacity values of 120 mg Trolox/100g, 142.50 mg Trolox/100 g, and 202.50 mg Trolox/100 g, respectively (Table 1., Figure 5).

Table 2. Mineral contain of Chinese jujube fruits

Genotype	Pb µg/kg	Cd µg/kg	Cr µg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg	Na mg/100 g	K mg/100 g	Ca mg/100 g	Mg mg/100 g
Jurilovca I 1	7.88	<1	164.18	0.9	2.9	**2.89**	**1.28**	22.61	42.09	**38.95**	**10.99**
Jurilovca I 2	13.53	<1	137.96	0.99	1.96	2.51	0.38	22.95	**51.99**	**37.51**	**11.06**
Jurilovca III 1	16.8	<1	**242**	**1.98**	**3.85**	**3.77**	0.7	26.08	**53.61**	**47.95**	**11.9**
Mahmudia 1	**49.86**	**3.87**	102.2	0.76	2.59	**4.06**	0.13	25.42	**55.16**	**34.66**	**11.56**
Mahmudia 2	9.21	<1	63.4	1.47	**3.72**	**4.03**	0.39	26.78	51.06	**36.3**	**11.94**
Ostrov	**45.43**	**3.66**	<1	1.54	2.21	2.48	0.22	26.41	**52.54**	14.44	9.46
Bugeac	8.9	<1	**543.6**	0.6	2.48	**3.39**	0.2	19.51	47.87	**25.09**	**10.68**
Taigu Ban	**26.73**	2.17	130.39	**1.89**	**6.28**	2.04	**1.24**	**32.02**	**53.6**	9.44	8.21
Hu Ping	17.68	1.89	63.46	**3.14**	1.39	1.93	0.5	**33.18**	49.43	6.29	7.78
Xuan Chen Jiang Zao	20.77	2.58	112.38	1.49	2.79	1.52	0.49	24.55	**54.07**	9.57	8.78
Jun Zao	17.37	2.3	<1	**1.97**	2.93	2.24	**1.42**	**32.39**	**51.88**	8.33	7.85
Hongan	**40**	2.2	**188.6**	**1.86**	2.28	2.08	**1.89**	**30.35**	49.79	9.97	8.47

The highest lead content was recorded on Mahmudia 1 and Ostrov genotypes with values of 49.86 µg/kg and 45.43 µg/kg. In contrast, in the Chinese jujube varieties, the highest values were found in the Taigu Ban variety, with a weight of 26.73 µg/kg, and the Xuan Chen Jiang Zao variety, with a value of 20.77 µg/kg. According to Commission Regulation (EC) No. 1881/2006 on maximum levels for specific contaminants in food products, the permissible limit for lead content in fruits is 0.1 mg/kg, equivalent to 100 µg/kg (Table 2).

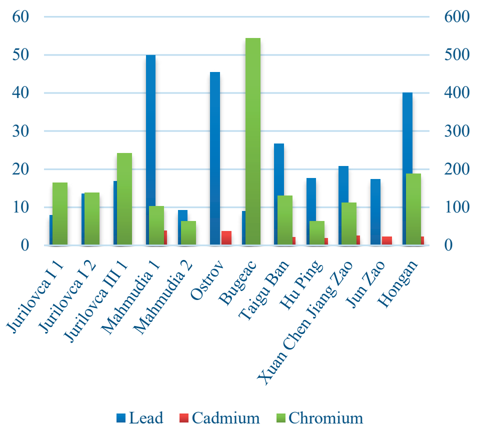

Figure 6. Content of lead, cadmium, and chromium (µg/kg) from jujube fruits

Cadmium is another heavy metal that can be toxic to the human body and can be found in some fruits and vegetables, especially those grown in contaminated soils or exposed to other sources of pollution such as water or air. Generally, fruits with thicker skins, such as bananas, oranges, and grapefruits, tend to have lower cadmium content than fruits with thinner skins, such as apples or grapes. In our case, the highest values were recorded in the fruits of Mahmudia 1 and Ostrov genotypes, with values of 3.87 µg/kg and 3.66 µg/kg. In the Chinese jujube varieties, the highest values were found in the fruits of Xuan Chen Jiang Zao and Jun Zao varieties, with values of 2.58 µg/kg and 2.30 µg/kg, respectively (Figure 6). According to Commission Regulation (EC) No. 1881/2006 on maximum levels for specific contaminants in food products, the permissible limit for cadmium content in fruits is 0.05 mg/kg, equivalent to 50 µg/kg. Chromium is an essential trace element for the human body and can be found in many foods, including fruits and vegetables. However, excessive consumption of chromium can be toxic to the body. In our case, the highest values in *Ziziphus jujube* genotypes were recorded in the Bugeac and Jurilovca III 1 genotypes, with 543.64 µg/kg values and 242.0 µg/kg values. On the Chinese jujube varieties, the highest values were found in Hongan and Taigu Ban varieties, with values of 188.59 µg/kg and 130.39 µg/kg (Table 2.).

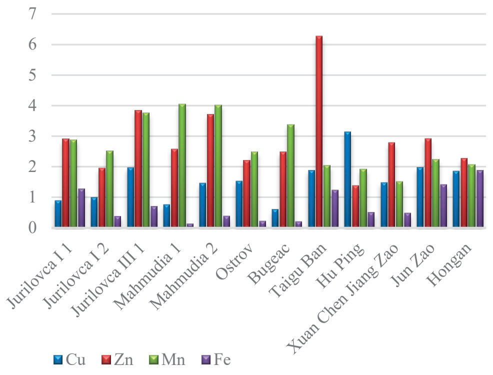

Figure 7. Content of Cu, Zn, Mn and Fe from jujube fruits (mg/kg)

Copper is an essential mineral for human health, involved in various physiological processes such as red blood cell formation and energy production. However, excessive copper consumption can be toxic and adversely affect health. In this case, the highest values recorded in Chinese jujube genotypes were found in the Jurilovca III 1 and Ostrov genotypes, with values of 1.98 mg/kg and 1.54 mg/kg, respectively. In the analyzed Chinese jujube varieties, the highest values were found in the Hu Ping and Jun Zao varieties, with values of 3.14 mg/kg and 1.97 mg/kg (Table 2, Figure 7).

Zinc is an essential mineral for human health and can be found in many foods, including some fruits. Zinc plays a vital role in the functioning of the immune system, wound healing, and average growth and development of the body. In this case, the highest zinc content was recorded in the Jurilovca III 1 and Mahmudia 2 genotypes, with values of 3.85 mg/kg and 3.72 mg/kg, respectively. In the analyzed Chinese jujube varieties, the highest values were found in the Taigu Ban and Jun Zao varieties, with values of 6.2 mg/kg and 2.93 mg/kg (Table 2, Figure 7).

Manganese is an essential mineral for the human body and is necessary for the normal functioning of many enzymes and metabolic processes. Manganese can be found in many foods, including fruits such as bananas, berries, and pineapple. In this case, the highest content recorded in Chinese jujube genotypes was found in the Mahmudia 1 and Mahmudia 2 genotypes, with values of 4.06 mg/kg and 4.03 mg/kg, respectively. In the analyzed Chinese jujube varieties, the highest values were identified in the Jun Zao and Hongan varieties, with values of 2.24 mg/kg and 2.08 mg/kg (Table 2, Figure 7).

Iron is an essential mineral for the human body and is necessary for producing hemoglobin in the blood, which carries oxygen to the cells. Iron can be found in many foods, including some fruits such as plums, figs, and raisins. The highest iron content in our case was recorded in the Jurilovca I 1 and Jurilovca III 1 genotypes, with values of 1.28 mg/kg and 0.70 mg/kg, respectively. In the analyzed jujube varieties, the highest values were found in the Hongan and Jun Zao varieties, with values of 1.89 mg/kg and 1.42 mg/kg (Table 2, Figure 7).

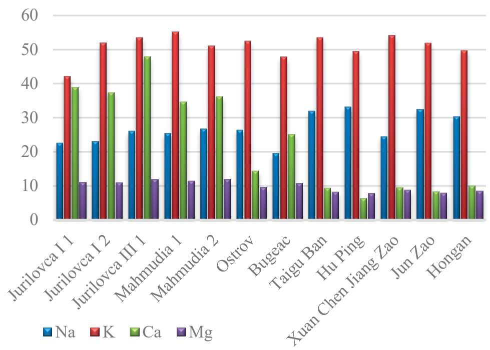

Figure 8. Content of Na, K, Ca and Mg from jujube fruits (mg/kg)

The genotypes with the lowest sodium content were Bugeac, Jurilovca I 1, and Jurilovca I 2, with values of 19.51, 22.61, and 22.95 mg/100g, respectively. On the other hand, the analyzed varieties, Hu Ping, Jun Zao, and Taigu Ban, had higher sodium content with values of 33.18, 32.39, and 32.02 mg/100 g, respectively (Table 2, Figure 8). Generally, the sodium levels in these genotypes varied significantly, which can be important when selecting or choosing between different Chinese jujube varieties based on their sodium content. High sodium content may be associated with specific health issues like hypertension.

A significant variation in potassium content can be observed among genotypes, ranging from 42.09 mg/100 g to 55.16 mg/100 g. The highest potassium levels were found in the genotypes Mahmudia 1, Jurilovca III 1, and Ostrov, with 55.16, 53.61, and 52.54 mg/100g values. In contrast, the lowest levels were recorded in Jurilovca I 1, Bugeac, and the Hu Ping variety, with 42.09, 47.87, and 49.43 mg/100 g values, respectively (Table 2, Figure 8).
Calcium is an essential mineral for the human body, involved in various physiological functions such as maintaining bone and teeth health, nerve impulse transmission, muscle contraction, and blood clotting. A calcium-rich diet can reduce the risk of osteoporosis and bone fractures in advanced age.
The calcium content in these genotypes varied significantly, ranging from 6.29 mg/100 g to 47.95 mg/100 g. Genotypes with the highest calcium values were Jurilovca III 1, Jurilovca I 1, and Jurilovca I 2, with values of 47.95, 38.95, and 37.51 mg/100 g, respectively. On the other hand, the lowest values were recorded in the Hu Ping, Jun Zao, and Taigu Ban cultivars, with values of 6.29, 8.33, and 9.44 mg/100 g, respectively (Table 2, Figure 8).
Magnesium is an essential mineral for health, involved in several critical metabolic processes such as protein and DNA synthesis, nervous and muscular system function, and blood glucose regulation. Additionally, magnesium plays a crucial role in maintaining bone and heart health.
It can be observed that all genotypes had a magnesium content of around 10 g/100 g. However, there is a slight variation among genotypes, with the highest values found in the genotypes Mahmudia 2, Jurilovca III 1, and Mahmudia 1, with values of 11.94, 11.90, and 11.56 g/100 g, respectively. Meanwhile, the lowest values were present in the Hu Ping, Jun Zao, and Taigu Ban varieties, with values of 7.78, 7.85, and 8.21 g/100 g, respectively (Table 2, Figure 8).

CONCLUSIONS

The analysis of the biochemical composition of Chinese jujube fruits revealed their significant abundance in sugar content, dry matter, polyphenols, and antioxidant capacity. Among the studied genotypes, Jurilovca III 1 showed higher values than most genotypes, with Mahmudia 2 following closely.
As for mineral content, among the studied genotypes again, Jurilovca III 1 showed higher values for more minerals than others, followed by Mahmudia 1 this time.
All fruits from the wild genotypes, except for Ostrov, present higher potassium, calcium, and magnesium values than those of the analyzed varieties.

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INFLUENCE OF PHOTOSELECTIVE PROTECTIVE NETS ON THE SENSORY CHARACTERISTICS OF FRUITS OF THE PINOVA APPLE CULTIVAR

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Abstract

Changing the light regime during the growing season by using photoselective nets in devices protecting against hail can have an impact on the yield and its quality. The white and black nets used do not change the spectral composition of the light passing through the grids, but act as shades, reducing the amount of light that passes through the grids. The influence of this effect on the sensory characteristics of Pinova apple fruits was investigated in an orchard with a support structure with an anti-hail net located in Northern Bulgaria. The sensory evaluation of the Pinova cultivar shows that the fruits under the most commonly used black net in orchards do not differ significantly in taste, aroma, and consistency from those grown under white, yellow, and red coverings. Only the influence of different types of nets on the appearance of the fruits of the Pinova apple cultivar has been statistically proven..

***Key words**: Pinova, photoselective net, sensory analysis, appearance, color, taste and aroma.*

INTRODUCTION

The use of photoselective nets in modern fruit growing is increasingly being applied as a safe alternative to successfully address the challenges of various stressors threatening fruit production.

Net systems are usually used to protect orchards from hail. Today, they are also designed to screen spectral bands of solar radiation and transform some of the direct light into diffuse. This process controls the physiological changes occurring in the plants and can direct them in the direction desired by the producer related to fruit quality (Raveh et al., 2003).

Photoselective nets, through their ability to scatter light, can improve the efficiency of their use by fruit plants (Meena et al., 2016). According to Sivakumar et al. (2017) this would change metabolic processes and positively affect the retention and development of quality fruits.

The emerging new microclimate under the nets affects fruit color and flesh quality differently. This is the reason for the different relationship between plants and the environment in areas without nets and those covered with nets (Bosco et al., 2015; Brglez Sever et al., 2015; Dussi et al., 2005; Reay et al., 1998).

In studies on the influence of nets on fruit flesh quality, parameters such as firmness, ripeness, sugar content, and acidity were less affected than parameters determining the appearance of the fruit - size and color. The firmness of the fruit flesh under the differently colored nets is different. Differently colored photo selective nets provoke different reactions regarding the flesh of the fruit species. According to Giaccone et al. (2012) covering with white nets increased fruit sugar content, in contrast to red ones. Other authors did not indicate differences in the acidity of the fruits of trees covered with differently colored nets (Ordóñez et al., 2016).

Sunburn is one of the factors that can reduce the quality of fruits. It can cause a 10-50% reduction in apple fruit yield (Wünsche et al., 2001; Kalcsits et al., 2017). Some cultivars are very susceptible to these damages (Dussi et al., 2005). Black nets are more effective in shading and reducing burn rates compared to white nets (Amarante et al., 2011).

Safety nets in fruit growing are constantly being improved. Attention is paid to their characteristics affecting the quality of fruit production. Research on the impact of photo selective nets on the physicochemical and sensory quality characteristics of fruits in apple cultivars is insufficient. In our experiment, we set ourselves the task of studying them in the Pinova cultivar. Pinova apple cultivar was obtained in Germany by crossing Clivia and Golden Delicious performed at the Institute of Fruit Growing in the city of Pilnitz. The ripening period is in the last ten days of September (Lichev et al., 2012). The aim of the present work is to evaluate the influence of photoselective nets on the sensory characteristics of Pinova apple fruits based on the overall sensory evaluation and the average evaluations for the indicators of appearance, color, consistency, aroma, and taste.

MATERIALS AND METHODS

The physico-chemical and sensory quality characteristics of apple fruits of the Pinova cultivar, grafted on M9 T337 rootstock and grown in an apple orchard located in Northern Bulgaria, were studied. The planting density is 250 plants per decare. A sod-mulch system is applied to maintain the soil surface and trees are drip irrigated. The anti-hail net system has been built in white, yellow, red, and black colors.

The analyzes were carried out in the Food Testing Laboratory at the Institute of Food Preservation and Quality - Plovdiv using standardized methods and adapted methodologies.

Five fruits per variant were obtained on the day of harvest and stored for one day at 20 ± 5°C until the sensory evaluation.

When assessing the quality of Pinova apple fruits, a descriptive method was used with a specific description of the sensory indicators appearance, color, consistency, taste and aroma depending on the characteristics of the cultivar and according to Commission Regulation (EU) No. 1580/2007 of December 21, 2007 to determine the rules for the implementation of Council Regulation (EU) No. 2200/96, (EU) No. 2201/96 and (EU) No. 1182/2007 in the fruit and vegetable sector (Table 1). The advantage of this method is that it is carried out by experienced professionals.

The analyzes were carried out in a training room by a committee including five trained experts, who were provided with apple fruits, as coded samples of the four growing variants and the specific descriptions of the indicators with weighting factor respectively for appearance - 0.30; colour - 0.20; consistency - 0.20; taste - 0.20 and aroma - 0.10.

Table 1. Description of the studied sensory indicators of apple fruits, cultivar Pinova

Parameters	Description
Appearance - Fruit shape; - Size Skin color Strength of fruit skin	Globose-conical, regular with a smooth surface; Handle and calyx fossa - wide and deep, without specific features. Medium to large, according to the size in Commission Regulation (EU) No. 1580/2007 of December 21, 2007, the fruits belong to group L Medium to large 150 to 300 grams - Section diameter: = extra - 60mm; = 1st quality - 55 mm. = 2nd quality - 50 mm - Weight: = extra 90 g = 1st quality - 80 g = 2nd quality - 70 g According to the coloring in Commission Regulation (EU) No. 1580/2007 of December 21, 2007, Pinova cultivar belongs to group "C" - cultivars colored in stripes, but weakly colored. The general surface of the fruit with a pale red coloration/slight reddening or streaking coloration or yellow-green color with red-orange fuzzy color or striped cover color covering 90% of the fruit or yellow main color and bright red covering color covering 70% of the fruit in stripes for; = "Extra" - 1/3; = 1st quality - 1/10. Strong, thick, and shiny
Color of fruit flesh	Light yellow to a yellowish tinge
Consistency	Juicy, with rather rough cells
Taste	Thick, sweet-sour or pleasantly sweet - sour
Aroma	Fine aroma to aromatic, strongly aromatic

The evaluation was carried out terminologically, then quantified on a five-point hedonic rating scale, in which these indicators are present in the considered sample, by awarding points or by constructing graphs.

All fruits in the quality determination group were measured for weight, height, and width. Fruit weight was determined with a laboratory technical balance A200 S, expressed in grams (g). Fruit height and width were measured with a digital caliper, values were expressed in

millimeters (mm). The data were averaged for each variant.
The physicochemical indicators were determined: the soluble dry matter - BDS 17257:1991, the content of titratable acidity - BDS 6996:1993, and the content of total sugars - BDS 7169:1989 to determine the relatively objective state of the fruits and to facilitate the trained tasters to interpret the sensations for taste, sweetness, and acidity.

Mathematical and statistical processing

Results presented are arithmetic means of at least three parallel determinations, with coefficients of variation less than 5%. The statistical processing of the data was carried out with the STATISTICA and ANOVA programs, Microsoft Excel.

RESULTS AND DISCUSSIONS

The appearance and sensory qualities of the flesh of the fruit are important for the acceptance of a cultivar, as they influence consumer choice. Figure 1 shows the average evaluation of the experts for the appearance indicator. The committee of experts evaluated the appearance of apple fruits from all growing variants of Pinova cultivar with scores above 4.45 in the description of the attractiveness of appearance, taking into account the shape, uniformity, size, and background color of the coating. The description of the indicator characteristic of the cultivar is that the fruits are uniform, globular-conical in shape, medium-large in size with an average weight over 170.00 g, a diameter of the section over 70 mm, and a smooth surface.

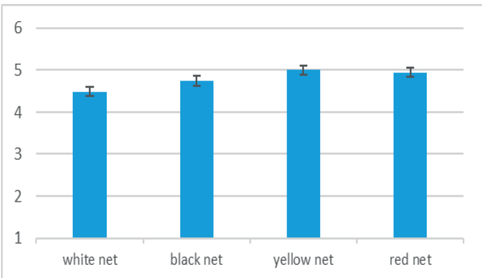

Figure 1. The appearance of fruits of cultivar Pinova grown under different photoselective nets

The experts defined descriptions of the skin color of the apple fruits on the differently colored networks and gave average ratings above 4.5 (Figure 2).

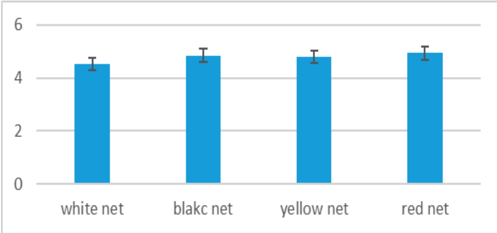

Figure 2. Color of fruits of cultivar Pinova grown under different photoselective nets

The maximum score for fruit skin color was given for fruit grown under the red net (4.95) (Figures 2). The fruits have a yellow-green main color with small brown lenticel dots and red-orange vertical stripes covering 90% of the fruit (Figure 3).

Figure 3. Apple fruits of Pinova cultivar, grown under the red net

Apple fruits grown under the black and yellow net did not have a statistically distinguishable mean skin colour score (4.85 and 4.80, respectively) (Figure 2).
The skin colour of fruit grown under the black net is mainly yellow-green with small brown lenticel dots, red vertical stripes fuzzy over 90% of the fruit surface, and pale cream dots (Figure 4).

Figure 4. Apple fruits of Pinova cultivar,
grown under the black net

Fruits grown under the yellow net are primarily yellow with brown lenticel dots, with red vertical stripes diffused over 2/3 or 70% of the fruit surface and pale cream dots (Figure 5).

Figure 5. Apple fruits of Pinova cultivar,
grown under the yellow net

Fruits grown under the white net are yellow main colour with brown lenticel dots, with red colored vertical stripes covering 1/3 to 2/3 better percentage of the fruit surface with pale cream dots (Figure 6). Experts rated the fruits of this variant with a relatively lower average rating of 4.54 (Figure 2).

Figure 6. Apple fruits of Pinova cultivar, grown under
the white net

The flesh of the fruits of the white and red net variants is slightly yellow-cream colored, and of the other two net variants, yellow and black, the flesh is cream-colored.

Regarding the consistency indicator, the committee rated all fruit variants with high average scores above 4.7 (Figure 7) as statistically indistinguishable. All apple fruits are technologically ripe, the skin is strong, thick, and shiny. The experts defined the fruit flesh of all variants as firm with rough cells and juicy. In the case of apple fruits grown under the yellow net, the commission found juicier fruit flesh, which is separated from the fleshy part when compressed when chewing. There is no significant influence of differently colored photoselective networks on the quality indicator consistency.

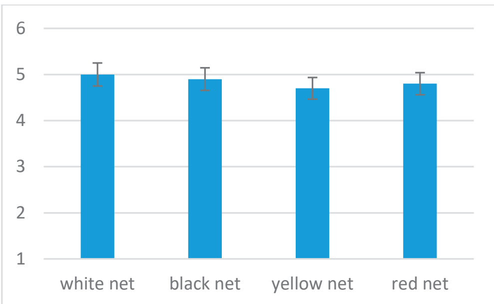

Figure 7. Consistency of fruits of cultivar Pinova grown
under different photoselective nets

Regarding the determination of the quality of apple fruits, not unidirectional opinions can be found in the literature in terms of which indicators dominate. Important indicators of quality are color, size, and wax cover (Hansen, 2003), recent studies on taste indicators show that many consumers choose apple fruits based on taste, aroma, and other intrinsic quality attributes (Harker, 2002; Moxham, 2003).
Research was carried out to determine soluble dry matter and the results showed that all apple fruits have dry matter of about 15.0%, with the same values for the fruits grown under white and black net 15.6%, with the highest value being the fruits under the red net 15.7% and with the lowest 15.0% the fruits obtained under the yellow net.
The sugar coefficient varies from 83% for fruit under the black net to 88% for fruit under the white net. The values for this indicator are the same for fruits below the yellow and red nets - 85%. The sugar-acid ratio is the lowest for fruits under the black net -12.76% and the highest for fruits under the white net - 19.13%. The fruits under the yellow and black nets occupied intermediate position in this parameter with 17.70% and 14.65%, respectively.
These data on the indicators cited above helped the experts in the interpretation of the sensations.
The committee assessed the taste of all apple fruits as thick and juicy, with average scores above 4.5 (Figure 8).

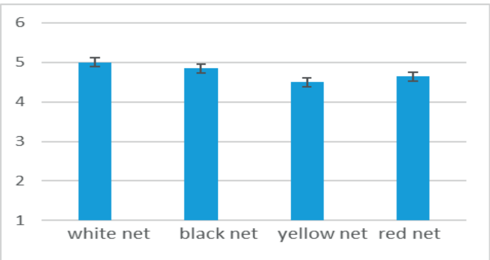

Figure 8. Taste of fruits of cultivar Pinova grown under different photoselective nets

A linear relationship with an average coefficient of determination $R^2 = 0.59$ was found between the evaluations of the taste and consistency indicators. For the rest of the sensory indicators, such dependencies were not established.
The fruits grown under white and black nets have a medium sweet to sweet taste. A maximum score of 5 given by the experts was obtained for fruits under the white net, which confirms the conclusion of Giaccone et al. (2012). A positive correlation was established between the sensory-determined taste and the analyzed soluble dry matter and sugar coefficient of the fruits. Apple fruits grown under the yellow net have a sweet-sour taste, and under the red mesh, they have a sweet taste. The fruits grown under the yellow net -4.50 are rated lower. To form the sweetness and aroma of apple fruits, the plants need high temperatures and constant sunshine. Since apple fruits rarely develop their full aroma immediately after harvest, fruit aroma is defined as slightly aromatic to aromatic (Figure 9).

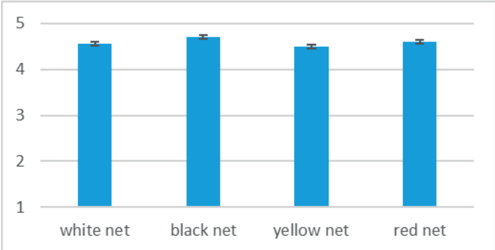

Figure 9. Aroma of fruits of cultivar Pinova grown under different photoselective nets.

The experts rated the fruits grown under the red and black nets as having aromatic intensity and the fruits under the yellow and white nets as having low aromatic intensity.

The general sensory evaluation containing all indicators of the apple fruits from the studied four variants is presented in Figure 10. All apple fruits have a total sensory score above 4.5. Fruits grown under the red and black net have a rating of 4.8, and those grown under the white and yellow net 4.7.

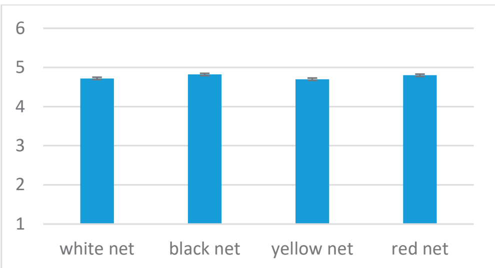

Figure 10. Summary sensory evaluation of fruits of cultivar Pinova grown under different photoselective nets

CONCLUSIONS

A study was conducted to evaluate the influence of photoselective nets on the sensory characteristics of apple fruits of Pinova cultivar based on the overall sensory evaluation and the average evaluations of the appearance, colour, consistency, aroma, and taste indicators.

The average ratings of apple fruits according to the studied indicators can be summarized as follows:

- apple fruits grown under a white net have maximum marks in terms of consistency, taste, the content of soluble dry substances, and sugar coefficient;
- the fruits grown under a yellow net have maximum marks in terms of appearance, weight, and size;
- the fruits under the red net have the highest scores for the colour indicator;
- the apple fruits under the black net have the highest score in terms of aroma and general sensory evaluation.

Summary sensory scores were statistically indistinguishable.

From the analysis of variance conducted at a significance level $\alpha = 0.05$ to establish the influence of the colour of the photoselective nets on the sensory indicators of the studied apple cultivar, it was found that the factor of the colour of the net affects the indicator of the appearance of the fruits.

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CONTRIBUTION TO THE KNOWLEDGE OF THE AUCHENORRHYNCHA FAUNA ASSOCIATED WITH APPLE AND PLUM ORCHARDS IN THE SOUTHERN PART OF ROMANIA IN 2022

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Abstract

The suborder Auchenorrhyncha is a diverse group of sap-feeding insect species, many of them of economic importance. The paper presents comparative results on the ecological characteristics of species communities of Auchenorrhyncha sampled in one apple orchard and one plum orchard that belong to the experimental field Moara Domnească Didactic Station in the year 2022. The insects were collected using dual-sided yellow sticky traps. A number of 9 traps/orchard was used from early April till mid-November (234 traps in total), replaced every two weeks. Altogether, 34 species have been identified, 30 in apple orchard and 32 in plum orchard, totaling 7403 specimens, 6058 (81.8%) in the apple orchard and 1345 (18.2%) in the plum orchard. The most abundant species found in the apple orchard was Empoasca decipiens with 3614 specimens (59.66%), with a constancy of 64.1%. In the plum orchard, two species were the most numerous, Fieberiella florii with 411 specimens (30.6%) and Zygina flammigera with 304 specimens (22.6%), having a constancy in the samples of 24.8 and 29.9% respectively. Adult populations dynamics were performed for relevant species in both orchards.

Key words*: apple and plum orchards, invasive insects, leafhoppers, planthoppers.*

INTRODUCTION

Apple and plum orchards are among the most important sources of food worldwide. They are affected by various pests and diseases, including insects from the suborder Auchenorrhyncha, also called “true hoppers”. There are over 42,000 described species of true hoppers characterized by insects that feed on plant sap and they can use the phloem, xylem and mesophyll as a food source, depending on the species (Mifsud et al., 2010). Through their way of feeding, by using specialized mouthparts that pierce and suck on plant sap, they can substantially damage the tissues of the attacked plants or be involved in the transmission of phytopathogens such as viruses, bacteria and phytoplasmas. According to Guglielmino et al. (2000), 73% of Auchenorrhyncha species are considered polyphagous, while the rest are oligophagous located mainly on woody plants. More than 150 species of true hoppers have been described as vectors of economically important phytopathogens (Purcell & Almeida, 2005; Resh & Carde, 2009). They can also secrete toxic substances while feeding on plant sap, causing abnormal development, damage or even death of the tissues and cells of the attacked plants (Karavin et al., 2021). On the other hand, a large number of true hoppers constitute considerable components of food webs in various ecosystems (Schmidt-Entling & Siegenthaler, 2009).

More importantly, many members of the Auchenorrhyncha suborder have acquired the status of invasive species, which, in addition to their economic impact on native vegetation and crops, can also be a threat to native biodiversity (Roques et al., 2009). In the European continent, several authors have identified different invasive species of true hoppers, with a total of 32, most of them with origins in North America and East Asia (Mifsud et al., 2010; Gjonov & Shishiniova, 2014; Šćiban & Kosovac, 2020).

Because of their economically important status, there are different studies of the

Auchenorrhyncha fauna in Europe in various especially in the case of bacterial and phytoplasma vectors (Bleicher et al., 2006; Tedeschi & Alma, 2006; Ricci et al., 2009; Bleicher et al., 2010; Ayaz & Yücel, 2010; Elbeaino et al., 2014; Lopes et al., 2014; Grimová et al., 2016; Ben Moussa et al., 2016; Cornara et al., 2017; Tsagkarakis et al., 2018; Fischnaller et al., 2020; Thanou et al., 2020; Theodorou et al., 2021; Karavin et al., 2021). The only studies carried out in Romania concerning the Auchenorrhyncha fauna refer to their presence on apple, pear and plum in the Bucharest area (Cean & Cean, 2013; Chireceanu et al., 2019; Teodoru et al., 2021).

The purpose of this paper was to evaluate the species belonging to the Auchenorrhyncha suborder identified in one apple orchard and one plum orchard in the experimental field of Didactic Station in Moara Domnească in 2022 in term of the ecological characteristics and the adult population dynamic for the most abundant species.

MATERIALS AND METHODS

The study area consisted of two commercial orchards, apple and plum, that belong to the experimental field Moara Domnească Didactic Station (44°29'59.1"N/ 26°15'31.7"E, 81 m a.s.l) located at 15 km north-east from Bucharest in the southern part of Romania and the sampling took place in 2022. Both apple (22.5 ha) and plum (7 ha) orchards were established in 2005 and consisted in a mix of cultivars. The orchards were chemically treated for pests and diseases control, with the first treatment on March 1st and the last one on July 22nd. The orchards were neighboring with apricot and walnut orchards and sunflower and corn crops.

The insects were collected using dual-sided yellow optical sticky traps (A4 dimension) produced in Romania at the Chemistry Research Institute "Raluca Ripan" and placed in the canopy of trees. A number of 9 traps/orchard was used from early April till mid-November (234 traps in total), arranged diagonally across the orchards and replaced every two weeks. A SZ61 stereomicroscope types of orchards of fruit trees and shrubs, with camera mount was used for the identification of species in the Auchenorrhyncha suborder following morphological characteristics and identification keys found in literature (Dietrich, 2005; Biedermann & Niedringhaus, 2009; Mozaffarian, 2018; Karavin et al., 2021).

The ecological parameters of abundance (A), dominance (D%), constancy (C%) and ecological significance index (W%) of species communities of Auchenorrhyncha have been calculated using formulas in literature (Stan, 1994; Baban, 2006; Carmo et al., 2013). According to the values of the ecological parameters, species were classified as following: for dominance: subreceding species (D1 < 1%), receding species (D2 = 1-2%), subdominant species (D3 = 2-5%), dominant species (D4 = 5-10%), eudominant species (D5 > 10%); for constancy: accidental species (C1 = 1-25%), accessory species (C2 = 25-50%), constant species (C3 = 50-75%), euconstant species (C4 = 75-100%); for ecological significance index: accidental species (W1 < 1%), accessory species (W2 = 1-5%), characteristic species (W3 > 5%).

The population dynamic of adult insects was performed for the most abundant species in both orchards.

RESULTS AND DISCUSSIONS

In the year 2022, the Auchenorrhyncha complex in two studied orchards of apple and plum has been evaluated by comparing captured species on yellow sticky traps. A total of 7403 specimens were caught on yellow sticky traps in the April-November period, 2022 belonging to 34 species assigned to seven families of true hoppers, 30 in apple and 32 in plum (Table 1**)**. The identified captured species were represented by all the groups from the Auchenorrhyncha suborder, namely leafhoppers (Cicadellidae family), planthoppers (Cixiidae, Delphacidae and Flatidae families), treehoppers (Membracidae family), spittlebugs (Aphrophoridae family) and cicadas (Cicadidae family).

Table 1. Number of species and specimens captured in 2022 on apple and plum

Families	Apple		Plum	
	Specimens	Species	Specimens	Species
Cixiidae	128	3	85	3
Flatidae	3	1	2	1
Delphacidae	3	1	1	1
Cicadidae	1	1	3	1
Membracidae	5	1	2	1
Aphrophoridae	15	1	3	1
Cicadellidae	5903	22	1249	24
Total	**6058**	**30**	**1345**	**32**

Concerning the number of specimens, the apple orchard totaled a much larger number, namely 6058 (82%), 4.5 times higher compared with the plum orchard, where 1345 specimens (18%) were found. In another study in Romania concerning the Auchenorrhyncha fauna on apple and plum, similar results were found, with the abundance of adults on apple being five times higher comparative to plum (Teodoru et al., 2021). For both orchards, by far the most abundant family was Cicadellidae, with 5903 specimens on apple (97.44% of all specimens from apple) and 1249 specimens on plum (92.86% of all specimens from plum). The most numerous species were also found in the Cicadellidae family (Table 1), this result being found in many recent scientific papers on the fauna of Auchenorrhyncha in fruit tree and shrub orchards of Europe (Teodorescu, 2018; Tsagkarakis et al., 2018; Fischnaller et al., 2020; Thanou et al., 2020; Theodorou et al., 2021; Karavin et al., 2021; Teodoru et al., 2021). Regarding the Cicadellidae family, leafhoppers from the Typhlocybinae and Deltocephalinae subfamilies had the highest abundance in both orchards. The Typhlocybinae subfamily percentage was 83.06% in apple and 60.36% in plum, while the Deltocephalinae subfamily was 16.83% in apple and 39% in plum. Although there were much fewer specimens in the plum orchard, in the case of the Cicadellidae family, two more additional species were found compared to the apple orchard. However, these species were considered accidental, as they comprised only a few specimens.

During the monitoring period, the peak activity of total Auchenorrhyncha fauna reached values of 1031 specimens in early July-late August in the apple in relation to the plum orchard that reached 343 specimens in late September-mid-October (Figure 1). The smallest flight activity was in May-early June and late October-November for both orchards.

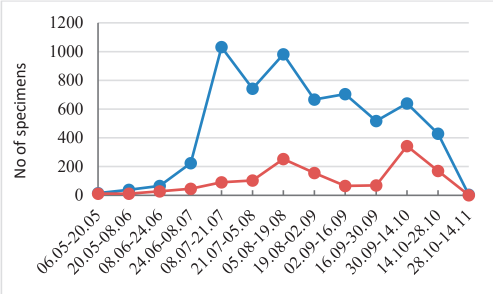

Figure 1. Auchenorrhyncha adults flight activity in the two orchards in 2022

Regarding invasive species and vectors of phytopathogenic organisms, 16 species were identified as confirmed and potential vectors of phytoplasma, totaling 77% in the apple orchard and 49% in the plum orchard (Table 2). The high abundance of phytoplasma vectors is explained by the fact that the family with the most numerous species of true hoppers is Cicadellidae, and most phytoplasma vectors belong to this family (Weintraub & Beanland, 2006; Wilson & Weintraub, 2007). An additional five species were identified as vectors of bacteria (two species) and viruses (three species). Among invasive species, seven species in our study were identified as new for the territory of Romania, with five of them playing an important role as phytoplasma vectors. Table 2 shows the list of invasive and/or vector species captured in the apple and plum orchards in 2022.

Table 2. Invasive and/or vector Auchenorrhyncha species identified in apple and plum in 2022

Auchenorrhyncha species	Invasive species/first report in Romania	Vector species - the transmitted phytopathogenic organism and the associated host plant	Authors
Cixiidae/Cixinae			
Cixius wagneri China, 1942	-	Confirmed vector: proteobacteria - Marginal Chlorosis of Strawberry (*Ca. Phlomobacter fragariae*) - strawberry	Danet et al., 2003
		Potential vector: proteobacteria - Disease Syndrome “Basses Richesses” (*Ca. Arsenophonus phytopathogenicus*) - sugar beet	Bressan et al., 2008
Hyalesthes obsoletus Signoret 1865	-	Confirmed vector: phytoplasma - stolbur (*Ca. Phytoplasma solani*) - grape vine, potato	Mitrović et al., 2016 Kosovac et al., 2019
Reptalus quinquecostatus Dufour, 1833	-	Potential vector: phytoplasma - stolbur (*Ca. Phytoplasma solani*) - grape vine	Pinzauti et al., 2008 Chuche et al., 2016
Flatidae/Flatinae			
Metcalfa pruinosa Say, 1830	Yes (2009)	Potential vector: phytoplasma - aster yellows (*Ca. Phytoplasma asteris*) - *Tagetes patula* (French marigold)	Grozea et al., 2011 Mergenthaler et al., 2020
Delphacidae/Criomorphinae			
Laodelphax striatellus Fallén, 1826	-	Confirmed vector: virus - Rice stripe virus - rice	Otuka et al., 2010
Membracidae/Smiliinae			
Stictocephala bisonia Kopp & Yonke, 1977	Yes (1955)	Potential vector: phytoplasma - apple proliferation (*Ca. Phytoplasma mali*) - apple	Popescu-Gorj, 1955 Duduk et al., 2008
Aphrophoridae/Aphrophorinae			
Philaenus spumarius Linnaeus, 1758	-	Confirmed vector: bacteria - *Xylella fastidiosa* - a wide range of agricultural crops and ornamental plants	Godefroid et al., 2022
		Potential vector: phytoplasma - apple proliferation (*Ca. Phytoplasma mali*) - apple, celery	Hegab & El-Zohairy, 1986
Cicadellidae/Aphrodinae			
Aphrodes makarovi Zachvatkin, 1948	-	Potential vector: phytoplasma – stolbur (*Ca. Phytoplasma solani*) - grape vine	Quaglino et al., 2019
		Potential vector: phytoplasma - Flavescence dorée (*Ca. Phytoplasma vitis*) - grape vine	Bressan et al., 2006
Cicadellidae/Agalliinae			
Anaceratagallia ribauti Ossiannilsson, 1938	-	Potential vector: phytoplasma - stolbur (Candidatus *Phytoplasma solani*) - broad bean	Riedle-Bauer et al., 2008
Cicadellidae/Typhlocybinae			
Empoasca decipiens Paoli, 1930	-	Confirmed vector: phytoplasma - Lime witches' broom (*Ca. Phytoplasma aurantifolia*) - lime, *Ranunculus* sp.	Parrella et al., 2008 Alhudaib et al., 2009
		Confirmed vector: phytoplasma - European stone fruit yellows (*Ca. Phytoplasma prunorum*) – apricot	Pastore et al., 2004
		Confirmed vector: phytoplasma - aster yellows (*Ca. Phytoplasma asteris*) - *Ranunculus* sp.	Parrella et al., 2008
Erasmoneura vulnerata Fitch, 1851	Yes (2018)	-	Chireceanu et al., 2020
Eupteryx atropunctata Goeze, 1778	-	Potential vector: phytoplasma - stolbur (*Ca. Phytoplasma solani*) - grape vine	Riedle-Bauer et al., 2006
		Potential vector: viroid - Potato spindle tuber viroid - potato, sunflower, beans	Patschke et al., 1997
Cicadellidae/ Deltocephalinae			
Fieberiella florii Stål, 1864	-	Confirmed vector: phytoplasma - Peach X-disease (*Ca. Phytoplasma pruni*) - apple	Krczal et al., 1988
		Potential vector: phytoplasma - apple proliferation (*Ca. Phytoplasma mali*) - apple	Tedeschi & Alma, 2006
		Potential vector: phytoplasma - European stone fruit yellows (*Ca. Phytoplasma prunorum*) - cherry	Landi et al., 2007
Anoplotettix fuscovenosus Ferrari, 1882	-	Potential vector: phytoplasma - Flavescence dorée (*Ca. Phytoplasma vitis*) - grape vine	Alma, 1995
Orientus ishidae Matsumura, 1902	Yes (2016)	Confirmed vector: phytoplasma - Flavescence dorée (*Ca. Phytoplasma vitis*) - grape vine	Alma et al., 2015 Chireceanu et al., 2017
		Potential vector: phytoplasma - Peach X-disease (*Ca. Phytoplasma pruni*) - celery	Davis et al., 2013
		Potential vector: phytoplasma – apple proliferation (*Ca. Phytoplasma mali*) - apple	Oppedisano, 2017
Phlogotettix cyclops Mulsant & Rey, 1855	Yes (2016)	Potential vector: phytoplasma - Flavescence dorée (*Ca. Phytoplasma vitis*) - grape vine	Strauss & Reisenzein, 2018 Chireceanu et al., 2020
Scaphoideus titanus Ball, 1932	Yes (2009)	Confirmed vector: phytoplasma - Flavescence dorée (*Ca. Phytoplasma vitis*) - grape vine	Chireceanu et al., 2011 Alma et al., 2015
Neoaliturus fenestratus Herrich-Schäffer, 1834	-	Confirmed vector: phytoplasma - stolbur (*Ca. Phytoplasma solani*) - grape vine, green salad, carrot	Orenstein et al., 2003 Mitrović et al., 2019
		Confirmed vector: phytoplasma - aster Yellows (*Ca. Phytoplasma asteris*) - grape vine	
Japananus hyalinus Osborn, 1900	Yes (1961)	-	Arzone, 1987
Psammotettix sp. Haupt, 1929	-	Confirmed vector: virus - Wheat dwarf virus - wheat, barley	Manurung et al., 2004
Allygidius atomarius Fabricius, 1794	-	Confirmed vector: phytoplasma - Elm yellows (*Ca. Phytoplasma ulmi*) - elm	Pavan, 2000

The four most abundant species in studied orchards, in descending order were *Empoasca decipiens* (59.66%), *Fieberiella florii* (11.18%), *Zyginidia pullula* (6.82%) and *Zygina flammigera* (6.54%) in the apple orchard (Figure 2) and *Fieberiella florii* (30.6%), *Zygina flammigera* (22.6%), *Zyginidia pullula* (15.46%) and *Empoasca decipiens* (9.22%) in the plum orchard (Figure 3).

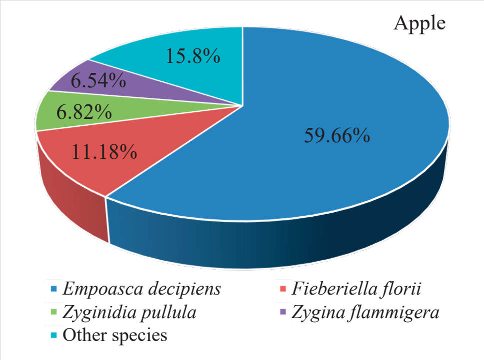

Figure 2. The abundance of the main species of true hoppers on apple

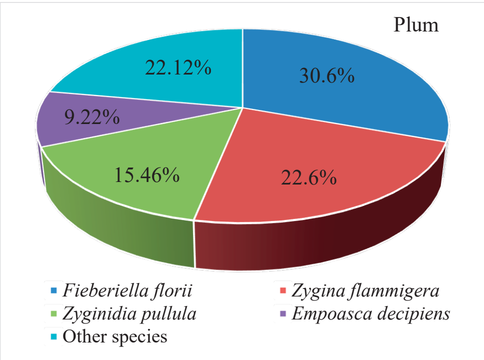

Figure 3. The abundance of the main species of true hoppers on plum

The abundance and the values of dominance, constancy and ecological significance of the two species communities, on apple and plum, are presented in Table 3.

By far the most specimens captured in total belonged to the species *Empoasca decipiens* (Cicadellidae, Typhlocybinae), which totaled 3738 specimens (50.5%), so more than half of all the specimens collected in both orchards (3614 on apple and 124 on plum). As for the ecological parameters, *E. decipiens* was found to be eudominant (D5), constant (C3) and characteristic (W3) in the apple orchard and dominant (D4) and accessory (C2 and W2) in the plum orchard. The second most numerous species was *Fieberiella florii* (Cicadellidae, Deltocephalinae), with 1088 specimens in total (14.7%), 677 on apple and 411 on plum. For the ecological parameters, on both apple and plum orchards, the species was identified as eudominant (D5), accessory (C2) and characteristic (W3). *Zygina flammigera* (Cicadellidae, Typhlocybinae) also had a considerable number of specimens (700), representing 9.45% (396 on apple and 304 on plum). The species was dominant (D4) and accessory (C2 and W2) in the apple orchard and eudominant (D5), accessory (C2) and characteristic (W3) in the plum orchard. *Zyginidia pullula* (Cicadellidae, Typhlocybinae), with 621 specimens (8.39%), 413 on apple and 208 on plum, was the fourth most numerous species. The species was dominant (D4) and accessory (C2 and W2) on apple and eudominant (D5), accidental (C1) and accessory (W2) on plum. After the fourth most abundant species (*Z. pullula*), each of the following species in descending order *Edwardsiana rosae*, *Reptalus quinquecostatus*, *Arboridia sp.*, *Orientus ishidae* and *Neoaliturus fenestratus* represented below 5% in terms of abundance. Of these, *O. ishidae* is invasive species and *R. quinquecostatus*, *O. ishidae* and *N. fenestratus* three are vectors of phytoplasmas.

Table 3. Ecological parameters for Auchenorrhyncha species captured in 2022

Taxa	Apple							Plum						
	A	D		C		W		A	D		C		W	
	(no)	Class	%	Class	%	Class	%	(no)	Class	%	Class	%	Class	%
Cixiidae/Cixinae														
Cixius wagneri China, 1942	36	0.59	D1	18.12	C1	0.1	W1	4	0.29	D1	2.56	C1	0.007	W1
Hyalesthes obsoletus Signoret 1865	1	0.01	D1	0.85	C1	0.00008	W1	8	0.59	D1	4.27	C1	0.02	W1
Reptalus quinquecostatus Dufour, 1833	91	1.5	D2	27.62	C2	0.41	W1	73	5.42	D4	17.94	C1	0.97	W1
Flatidae/Flatinae														
Metcalfa pruinosa Say, 1830	3	0.05	D1	2.56	C1	0.001	W1	2	0.14	D1	1.7	C1	0.002	W1
Delphacidae/Criomorphinae														
Laodelphax striatellus Fallén, 1826	3	0.05	D1	2.56	C1	0.001	W1	1	0.07	D1	0.85	C1	0.0005	W1
Cicadidae/Cicadettinae														
Dimissalna dimissa Hagen, 1856	1	0.01	D1	0.85	C1	0.00008	W1	3	0.22	D1	1.7	C1	0.003	W1
Membracidae/Smiliinae														
Stictocephala bisonia Kopp & Yonke, 1977	5	0.08	D1	3.41	C1	0.002	W1	2	0.14	D1	1.7	C1	0.002	W1
Aphrophoridae/Aphrophorinae														
Philaenus spumarius Linnaeus, 1758	15	0.24	D1	7.69	C1	0.01	W1	3	0.22	D1	2.56	C1	0.005	W1
Cicadellidae/Aphrodinae														
Aphrodes makarovi Zachvatkin, 1948	3	0.05	D1	1.7	C1	0.0008	W1	4	0.29	D1	1.7	C1	0.004	W1
Cicadellidae/Agalliinae														
Anaceratagallia ribauti Ossiannilsson, 1938	3	0.05	D1	2.56	C1	0.001	W1	3	0.22	D1	2.56	C1	0.005	W1
Cicadellidae/Eupelicinae														
Eupelix cuspidata Fabricius, 1775	-	-	-	-	-	-	-	1	0.07	D1	0.85	C1	0.0005	W1
Cicadellidae/Typhlocybinae														
Zyginella pulchra Löw, 1885	17	0.28	D1	8.54	C1	0.02	W1	3	0.22	D1	2.56	C1	0.005	W1
Empoasca decipiens Paoli, 1930	3614	59.65	D5	64.1	C3	38.23	W3	124	9.22	D4	33.33	C2	3.07	W2
Zygina flammigera Fourcroy, 1785	396	6.53	D4	47	C2	3.06	W2	304	22.6	D5	41.88	C2	9.46	W3
Erasmoneura vulnerata Fitch, 1851	48	0.79	D1	15.38	C1	0.12	W1	17	1.26	D2	9.4	C1	0.11	W1
Eupteryx atropunctata Goeze, 1778	18	0.29	D1	10.25	C1	0.03	W1	12	0.89	D1	5.98	C1	0.05	W1
Zyginidia pullula Boheman, 1845	413	6.81	D4	29.91	C2	2.03	W2	208	15.46	D5	14.52	C1	2.24	W2
Edwardsiana rosae Linnaeus, 1758	307	5.06	D4	29.05	C2	1.46	W2	31	2.3	D3	7.69	C1	0.17	W1
Arboridia sp.	90	1.48	D2	28.2	C2	0.41	W1	55	4.08	D3	17.94	C1	0.73	W1
Cicadellidae/ Deltocephalinae														
Fieberiella florii Stål, 1864	677	11.17	D5	47.86	C2	5.34	W3	411	30.55	D5	25.64	C2	7.83	W3
Anoplotettix fuscovenosus Ferrari, 1882	21	0.34	D1	9.4	C1	0.03	W1	4	0.29	D1	3.41	C1	0.009	W1
Orientus ishidae Matsumura, 1902	113	1.86	D2	23.93	C1	0.44	W1	7	0.52	D1	4.27	C1	0.02	W1
Phlepsius ornatus Perris, 1857	-	-	-	-	-	-	-	3	0.22	D1	0.85	C1	0.001	W1
Platymetopius rostratus Herrich-Schäffer, 1834	9	0.14	D1	6.83	C1	0.009	W1	-	-	-	-	-	-	-
Platymetopius major Kirschbaum, 1868	29	0.47	D1	15.38	C1	0.07	W1	3	0.22	D1	2.56	C1	0.005	W1
Phlogotettix cyclops Mulsant & Rey, 1855	8	0.13	D1	3.41	C1	0.004	W1	-	-	-	-	-	-	-
Scaphoideus titanus Ball, 1932	7	0.11	D1	4.27	C1	0.004	W1	1	0.07	D1	0.85	C1	0.0005	W1
Selenocephalus obsoletus Germar, 1817	-	-	-	-	-	-	-	2	0.14	D1	1.7	C1	0.002	W1
Neoaliturus fenestratus Herrich-Schäffer, 1834	102	1.68	D2	24.78	C1	0.41	W1	6	0.44	D1	3.41	C1	0.01	W1
Japananus hyalinus Osborn, 1900	3	0.05	D1	1.7	C1	0.001	W1	2	0.14	D1	1.7	C1	0.002	W1
Psammotettix sp. Haupt, 1929	14	0.23	D1	10.25	C1	0.02	W1	37	2.75	D3	12.82	C1	0.35	W1
Penthimia nigra Goeze, 1778	-	-	-	-	-	-	-	1	0.07	D1	0.85	C1	0.0005	W1
Allygidius atomarius Fabricius, 1794	3	0.05	D1	2.56	C1	0.001	W1	4	0.29	D1	2.56	C1	0.007	W1
Allygus modestus Scott, 1876	8	0.13	D1	6.83	C1	0.009	W1	6	0.44	D1	4.27	C1	0.02	W1

The population dynamic of adults for the most abundant species in both orchards insects was performed. The dynamics of *E. decipiens* is presented in Figure 4. In the apple orchard, *Empoasca decipiens* reached its maximum peak in the first half of July (953 specimens), with considerable populations during the following months, until October. In the plum orchard, population of this species was very low throughout the year, peaking at only 40 adults in August. *E. decipiens* is a common occurrence in orchards (Emam et al., 2020) and one of the most important pests in greenhouses in Europe (Tounou et al., 2003).

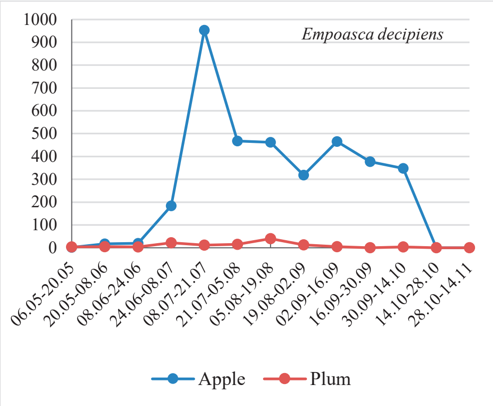

Figure 4. Population dynamics of *E. decipiens* in 2022

E. decipiens generally causes direct damage through feeding and the symptoms on host plants consist of chlorotic or necrotic areas also called "hopperburn" (Darwish, 2018). It is a highly polyphagous species and has been recorded on a wide variety of cultivated and spontaneous plants such as vines, tomatoes, cucumbers, potatoes, maize, beans, sesame and various ornamental plants (Raupach et al., 2002; Emam et al., 2020). In addition, this species has been identified as a potential vector of the following phytoplasmas: lime witches' broom (*Ca. Phytoplasma aurantifolia*) (Alhudaib et al., 2009), European stone fruit yellows (*Ca. Phytoplasma prunorum*) (Pastore et al., 2004) and aster yellows (*Ca. Phytoplasma asteris*) (Parrella et al., 2008).
The population dynamics of *Fieberiella florii* (Figure 5) reached one maximum peak, in the second half of October on apple (397 adults) and in the first half of the same month on plum (225 adults).As a pest species, it is one of the main vectors of peach X-disease (*Ca. Phytoplasma pruni*) on apple, especially in North America (Krczal et al., 1988). Tedeschi & Alma (2006) demonstrated using the molecular method that *F. florii* can be a potential vector of the apple proliferation phytoplasma (*Ca. Phytoplasma mali*), while Landi et al. (2007) detected European stone fruit yellows phytoplasma (*Ca. Phytoplasma prunorum*) in individuals captured in affected cherry orchards.

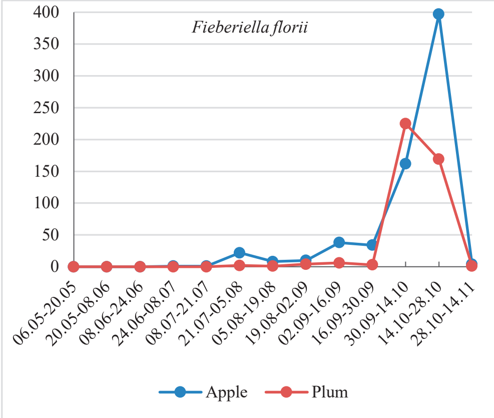

Figure 5. Population dynamics of *F. florii* in 2022

The population dynamics of *Zygina flammigera* (Figure 6) showed that in the apple orchard the species reached two peaks, the first between the end of July and the beginning of August (82 specimens) and another one, in the second half of August (99 specimens). In plum, the species reached one maximum peak only, in the first half of October (92 specimens).

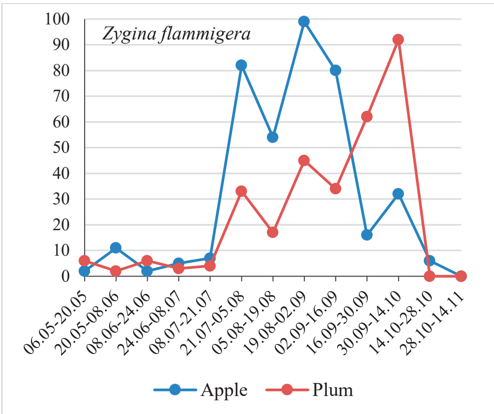

Figure 6. Population dynamics of *Z. flammigera* in 2022

Although this species has not been confirmed as a vector of phytopathogens, it can still cause direct damage through feeding on the sap of host plants. It is a polyphagous species that is common in fruit tree orchards (Teodoru et al., 2021). It has been reported as a minor pest in peach orchards of Italy (Viggiani et al., 1992) and Spain (Torres et al., 2000) but also as a major pest of peach and almond in Tunisia (Chaieb et al., 2011).

The population dynamics of *Zyginidia pullula* (Figure 7) showed that in both orchards, the species reached a single maximum peak in the first half of August, with 183 adults on apple and 157 adults on plum.

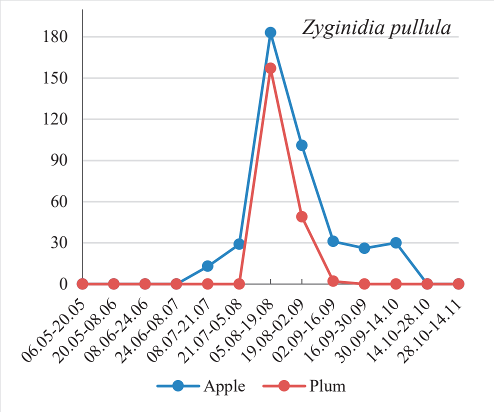

Figure 7. Population dynamics of *Z. pullula* in 2022

Like most of the leafhoppers from the Typhlocybinae subfamily, it causes direct damage to cultivated plants. It feeds on the mesophyll, so it directly affects the photosynthesis process of plants. This species is more common on grasses, including corn crops (Verzé & Mazzoglio, 1994; Negri et al., 2006).

CONCLUSIONS

Data on the Auchenorrhyncha fauna studied in two managed orchards of apple and plum in April-November 2022 showed that a total of 34 species were identified (30 on apple and 32 on plum) with a total of 7403 specimens (6058 on apple, 1345 on plum). The species belonged to a total of six families, namely Cixiidae, Flatidae, Delphacidae, Cicadidae, Membracidae, Aphrophoridae and Cicadellidae, with the later family being by far the most abundant in both apple (97.44%) and plum (92.86%) orchards. The species with the highest number of individuals were cicadellids *Empoasca decipiens*, *Fieberiella florii*, *Zygina flammigera* and *Zyginidia pullula*. *E. decipiens* alone was so abundant that it accounted more than a half of all the other species collected in both orchards (50.5%).

Of the 34 species captured on yellow sticky traps, 16 are cited in literature as phytoplasma vectors, 3 virus vectors and 2 vectors of bacteria. Moreover, seven species among them were identified as invasive in Europe and Romania: *Metcalfa pruinosa*, *Stictocephala bisonia*, *Erasmoneura vulnerata*, *Orientus ishidae*, *Phlogotettix cyclops*, *Scaphoideus titanus* and *Japananus hyalinus*.

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IDENTIFICATION OF GENETIC DIVERSITY AMONG SOME PEARS CULTIVARS WITH ISSR MARKERS

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Abstract

This study identified genetic diversity in pear cultivars using ISSR techniques. In breeding programs, molecular markers play an important role. Molecular markers are the most efficient tools for the study of taxonomy, genetic variability, phylogenetic analysis, gene tags, gene localization and development of new cultivars. ISSR techniques were used in the identification of the genetic diversity of 10 pear cultivars. Five out of the used primers in this study amplified clear and reproductible bands. The ISSR primers produced 51 bands, and 45 of them were polymorphic, with an average of 10.2 amplicons/primer. The size of the fragments varied from 200 to 1250 bp. The polymorphic bands, registered per primer ranged from 6 (844) to 11 (primer UBC 830). The percentage polymorphism was between 83.33 for UBC808 and 100% for primer 844. Degree of DNA polymorphism was estimated at 89.54% (ISSR). PIC registered values between 0.34 to 844 and 0.75 for UBC814. The primer 844 presented values of discrimination index (PI) 2.06. The obtained results will be useful to serve plant breeding programs.

Key words: *genetic analysis, ISSR markers, pear.*

INTRODUCTION

One of the most diverse and large plant families is the Rosaceae, comprising economically important fruit trees. This one the family consists of over 100 genera and 3,000 species and is considered the third plant family in terms of importance economic importance in temperate regions. (Zarei et al., 2017). The pear with up to 20 species in the world is a species belonging to the *Pyrus* genus of the Rosaceae family of the Rosales team. One of the most widely cultivated fruit crops in the world is the common pear (*Pyrus communis*). In the world, it is the most cultivated fruit species after the apple, (Ünal 2011). Today exist More than 5000 cultivars, although only a small percentage of them are cultivated commercially (Bell et al., 1996). The geographical distribution of pears can be divided into two main groups, including Asian and European pears (Koushesh-Saba et al., 2017; Kumar et al., 2017). European pears are located in Europe, North Africa, Iran, Central Asia, Asia Minor, and Afghanistan, with the most important species *P. communis*. Oriental pears such as the *P. serotina* synonym *P. pryifolia* distributed in Japan have originated from Eastern Asia (Arzani, 2017; Koushesh-Saba et al., 2017; Teng and Tanabe, 2004). Currently, pears are commercially cultivated in more than 50 temperate regions. Over the past decades, efforts have been made to evaluate the genetic diversity of Asian and European pears and other *Pyrus* species using biochemical, morphological, and molecular markers (Koushesh-Saba et al., 2017; Nikzad Gharehaghaji et al., 2014a).Molecular markers have been used for studying the genetic diversity, relationships, and origins of the cultivars, as well as for cultivar discrimination and fingerprinting of several fruit crops (e.g., Cervera et al., 1998; Dirlewanger et al., 1998; Fang and Roose, 1997; Gianfranceschi et al., 1998; Hokanson et al., 1998; Koller et al., 1993). Molecular markers have many advantages compared with phenotypic markers because they are stable, detectable in all tissues irrespective of growth, development and differentiation, and remain unaffected by fluctuations in environmental conditions, cultural impacts and pleiotropic effects (Gosal et al., 2010). For cultivar identification and taxonomic relationship studies in pears have already been used (Botta et al., 1998; Oliveira et al., 1999; Monte-Corvo et al., 2000) polymerase chain reaction (PCR)-based molecular markers such as randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990) and amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995). For genome studies inter-simple sequence repeat (ISSR) amplification is another microsatellite-based technique useful (Zietkiewicz et al., 1994). The study investigated at the molecular level some of the pear genotypes from different counties to obtain more information about their genetic relationships.

MATERIALS AND METHODS

A total of 10 pear cultivars belonging to three counties from West part of Romania were used in this study (Table 1).

Table 1. Biological material

Population	County	Population	County
1. Par rosu	Caras-Severin	6. Lubenicarka	Mehedinti
2. Lubinite	Caras-Severin	7. Par de Balvanesti	Mehedinti
3. Malaiete	Caras-Severin	8. Par de Malovat	Mehedinti
4. Albe de Sf.Petru	Timis	9. Mici galbene	Timis
5. Limunka	Mehedinti	10. Marganesc	Caras-Severin

DNA was isolated from young leaves using method of Doyle and Doyle (1990). The PCR reaction was performed with the following protocol: 42 ISSR cycles (95ºC/30s, 55ºC/30s, 72ºC/90s). Following amplification, the PCR products (10 μL) were loaded in 1.5% agarose gels, stained with ethidium bromide in Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0), and separated by electrophoresis, and photographed on an ultraviolet trans illuminator. Were selected for the analysis, only clear, repetitive DNA fragments. A dendrogram was constructed based on the similarity matrix. In view of the potential characterization of different molecular marker systems to evaluate inter population variability in the studied genotypes, different parameters were calculated:

- the total polymorphism generated by a certain primer (PIC = Polymorphic Information Content) which indicates its discriminatory power.

$$PIC = 1 - \sum_{i=1}^{n} P_{ij}^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 P_i^2 P_j^2$$

Pi - frequency of allele i; Pj - frequency of allele j; Pij - frequency of allele i for locus j; n - the total number of loci.

- the discrimination index (PI), which certifies the effectiveness of a particular primer in detecting polymorphism.

$$PI = \sum PIC$$

Genetic similarity among genotypes studied calculated through coefficient Jaccard, which was recommended to be used for dominant markers ISSR, RAPD, taking in view that the absence of a bands was associated to a homozygous locus. JC = a/(a + b + c), where a, b, c, represented the commons and un-commons of those genotypes (Dangi et al., 2004). On base of genetic similarity matrix among genotypes, it was made the dendrogram using the method of clusters average.

RESULTS AND DISCUSSIONS

As a result of reactions carried out with 5 ISSR primers, 45 polymorphic DNA fragments were obtained, their size varying from 200 to 1250 bp. The largest number of polymorphic fragments was produced in reactions with primers UBC 830, UBC 831 and UBC 808. The average of amplicons/primer was 10.2. The polymorphic bands, registered per primer ranged from 6 (844) to 11 (primer UBC 830). The percentage polymorphism was between 83.33 for UBC 808 and 100% for primer 844. Degree of DNA polymorphism was estimated at 89.54% (ISSR). These results were similar to the one presented by Monte-Corvo et al., 2001, where degree of DNA polymorphism was (79.5%). PIC registered values between 0.34 to 844 and 0.75 for UBC 814. The primer 844 presented values of discrimination index (PI)2.06.

Table 2. Polymorphism rate through ISSR primers

	Primer sequence 5'- 3'	No. of fragments amplifyed	Polymor-phic band	% polymor-phism	Pi	PIC $\overline{x} \pm s_{\overline{x}}$
UBC-831	(CT) 8T	11	10	90.9	4.54	0.454±0.044
UBC-808	(AG) 8C	12	10	83.33	4.1	0.41±0.05
UBC-830	(TG) 8G	13	11	84.61	5.08	0.462±0.049
UBC 814	(CT) 8A	9	8	88.88	6	0.75±0.068
844	(CT) 8RC	6	6	100	2.06	0.343±0.044
	Total	51	45	89.54		

Based on genetic similarity, pears populations were hierarchically classified into three clusters between which there is an average diversity of approximately 46%. The first group included four populations that possess approximately 65% of the common alleles of the five primers. The populations Par roşu and Mălăiețe, genetically similar to a degree of 72.55%, make up a first subcluster, while the populations Mici galbene and Mărgănesc differ from each other to a degree of approximately 31%. The second cluster is composed of the Limunka and Păr de Malovaţ populations, between which there is a genetic similarity of 61%. Between the populations of these two clusters, there is an average diversity of approximately 35%. The Lubinite, Albe de SFP, Păr de Bălvăneşti, and Lubenicarka populations represent a separate group that possesses approximately 65% of the common alleles of the five primers.

The interpopulation similarity (Table 3) for the alleles of the five ISSR primers had values ranging from 27.45% between Par de Malovat and Lubinițe to 78.43% between Par de Bălvăneşti and Albe de SfP.

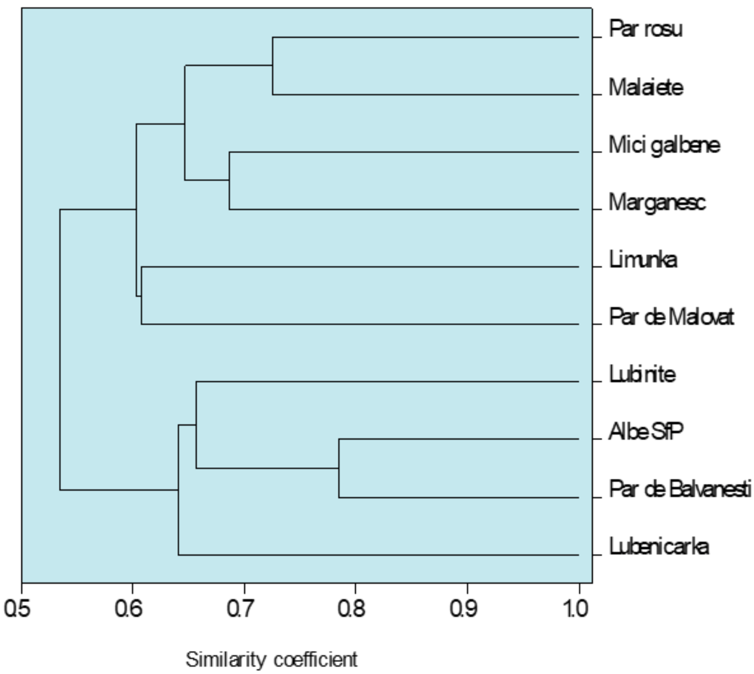

Figure.1. UPGMA clustering of pears population using ISSR primers

Table 3. Similarity matrix between pear cultivars using ISSR primers

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. Par rosu	1								
2. Lubinite	0.4314	1							
3. Malaiete	0.7255	0.4314	1						
4. Albe de Sf. Petru	0.6078	0.6275	0.5686	1					
5. Limunka	0.6078	0.4706	0.6471	0.4902	1				
6. Lubenicarka	0.6275	0.6471	0.6667	0.5882	0.4706	1			
7. Par de Balvanesti	0.6667	0.6863	0.5882	0.7843	0.5098	0.6863	1		
8. Par de Malovat	0.6078	0.2745	0.6863	0.4902	0.6078	0.4706	0.4314	1	
9. Mici galbene	0.5882	0.6471	0.6275	0.5882	0.549	0.5294	0.6078	0.5098	1
10. Marganesc	0.7059	0.5294	0.6667	0.5490	0.5882	0.6078	0.5686	0.6275	0.6863

Table 4. Analysis of variance for pear populations concerning the bands of ISSR primer

Population	Between groups		Within groups		F test
	SS	DF	SS	DF	
1. Par rosu	0.20	1	9.96	49	0.98
2. Lubinite	9.83	1	2.80	49	172.27**
3. Malaiete	0.50	1	10.48	49	2.36
4. Albe de Sf. Petru	0.95	1	10.03	49	4.63*
5. Limunka	0.16	1	12.00	49	0.64
6. Lubenicarka	1.30	1	10.63	49	5.98*
7. Par de Balvanesti	1.92	1	8.67	49	10.86**
8. Par de Malovat	4.32	1	7.83	49	27.05**
9. Mici galbene	1.97	1	9.37	49	10.29**
10. Marganesc	0.05	1	8.57	49	0.31

Regarding the populations being studied (Table 4), a notable contribution to the total variability regarding the spectrum of the different fragments amplified by the ISSR primers was observed in the case of the Lubinite and Par de Malovat populations, which is highlighted by a different allelic structure. The lowest variance values were recorded in the Mărgănesc and Păr roşu populations. The highest variability of the polymorphic bands within the first cluster was recorded in the Mălăieţe population, respectively in the Limunka population for the second cluster. Lubenikarka and Albe de Sf. Petru populations show a high influence on diversity at the level of the third cluster.

Table 5. Correlation coefficients between the similarity matrices given by different ISSR primer

Primer	UBC808	UBC830	UBC814	UBC844
UBC831	0.052 *p=0.733*	0.186 *p=0.22*	-0.091 *p=0.551*	0.418** *p=0.004*
UBC808		0.069 *p=0.648*	0.245 *p=0.105*	0.111 *p=0.466*
UBC830			-0.120 *p=0.432*	0.087 *p=0571*
UBC814				0.386** *p=0.009*

The low and statistically uncertain value of the correlation coefficient between the genetic similarity matrices identified using the different ISSR primers, attests that in general, the respective primers provide complementary information regarding the diversity of the analyzed pears populations.

CONCLUSIONS

The largest number of polymorphic fragments was produced in reactions with primers UBC 830, UBC 831 and UBC 808. The percentage polymorphism was between 83.33 for UBC 808 and 100% for primer 844. Degree of DNA polymorphism was estimated at 89.54% (ISSR). Based on genetic similarity, pears populations were hierarchically classified into three clusters between which there is an average diversity of approximately 46%. The first group included four populations that possess approximately 65% of the common alleles of the five primers. The second cluster is composed of the Limunka and Păr de Malovaţ populations, between which there is a genetic similarity of 61%. The Lubinite, Albe de SFP, Păr de Bălvăneşti, and Lubenicarka populations represent a separate group that possesses approximately 65% of the common alleles of the five primers. The obtained results with the ISSR analysis will be useful for plant breeding programs.

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EVALUATION OF OLD APPLE VARIETIES GROWN IN THE NORTH-EASTERN PART OF ROMANIA

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Abstract

Old apple cultivars, either originating from Romania or of foreign origin but grown widely in this region, were examined to provide a characterisation of the varieties. The aim of the thesis was to observe and evaluate the phenological, and morphological traits of the old apple cultivars using the following methods as: observation of flowering phenology traits and the classification of the cultivars into flowering time groups; characterisation of the gene bank collection and the determination of diversity using morphological markers; preparation of a number-coded characterisation based on the morphological and biological traits laid down in the UPOV guidelines. Identified and collected material provides the initial genetic source for variety improvement. Using the observations and determinations made, we identified the potential genitors for different useful characteristics that we used in controlled intra and interspecific hybridizing. Researchers constant concern refers to preserving, coplotting, periodically reorganizing and assessing the biological and agrochemical characteristics of each genotype, the exchange of biological material into the restricted manner and the assessment of the potential genitors for some useful characteristics used in genetic breeding programs.

Key words: *Malus, varieties, evaluation, diversity.*

INTRODUCTION

In the last years fruit consumption is becoming more and more varied and this trend leads to the necessity for a more various fruit assortment available on the market. This could be achieved in two ways: by re-introducing old varieties into cultivation, but also by using them as crossing partners in the development of new cultivars.
Despite the fact that the apple is the third cultivated species worldwide after citrus fruits and bananas, global apple production involves only a few dozen cultivars. This is understood as in the last decades the role of local cultivars has become completely insignificant.
Apple is a specie that is always vulnerable to natural disasters, pathogens or different pests. Eliminating this vulnerability can only be done when the maintenance and uninterrupted enrichment of the germoplasm field is achieved (Corneanu G. et al., 2020; Volk, G.M. et al., 2005; Bignami, C., et al., 2003).
In the limit situations, like climate change or appearance of new pathogen species, there could be reached an ecological catastrophe and this restricted range of cultivars could endanger the reliability and profitability of apple production as we know it.
In this scenario, the old apple cultivars have a highly significant value from biodiversity the point of view due to the good resistance or excellent inner quality, while others satisfy special consumer demands (Hammer K. et al., 2003).
Old varieties are a rich source of genetic source with a highly significant role in breeding, and some have very positive characteristics, which is very important in cultivation (Routson' K. J. et al., 2009).
The aim of the present study is to provide useful information for gene bank analysis and to achieve better knowledge of cultivar traits, which could be useful for scientists and growers.

MATERIALS AND METHODS

During 2019-2022 there was performed a study in an apple orchard that involved 12 old apple varieties, traditionally grown (table 1) located in Solca and Cacica villages, Suceava, North-East of Romania (Figure 1). The total number of analysed trees was 162.

Orchard was established during 1850-1900 and since then trees were constantly renewed.

Planting distances are 8 x 6 m. The average age of all trees in research was 40 years. Trees have been held under non-irrigated extensive cultural practice.

The aim of research was to observe and evaluate the phenological and morphological traits of the old apple cultivars using the following observation of flowering phenology traits and the classification of the cultivars into flowering time groups.

Figure 1. Location of the experience

Table 1. The list of studied varieties and location

Variety's name/ Abreviation	Location	Nr. of trees
'Trauben' (T)	Solca village - own orchard	4
'Bănățesc' (B)	Solca village - own orchard	2
'Domnesc' (D)	Solca village - own orchard	130
'Belle Fleur Jaune' (BFJ)	Solca village - own orchard	3
'Belle de Boskoop' (BB)	Solca village - own orchard	4
'Renet de Canada' (RC)	Solca village - own orchard	2
'London Pepping' (LP)	Solca village - own orchard	2
'Parmen auriu' (PA)	Solca village - own orchard	4
'Renet de Landsberg' (RL)	Solca village - own orchard	2
'Jonathan' (JO)	Solca village - own orchard	5
'Gravenstein' (G)	Cacica village	2
'Papirovka' (P)	Solca village - own orchard	2
TOTAL		162

Observations on flowering phenology were carried out in the years 2019–2022. Data were recorded every two days during the flowering period. The beginning of flowering, the peak flowering period and the end of flowering were determined by subjective observations (as laid down in the UPOV guidelines).

The first phase of the study consisted of data collection and centralization of information.

The second phase consisted of observations and data systematization.

There were used UPOV descriptors for apple (Batelja Lodeta Kristina et al., 2019).

The orchard (4.75 ha) is located on a plot of land with a profile and soil type of the black soil class. The cernisoil class includes soils where the diagnostic horizon is A, relatively soft, rich in calcium humus, almost exclusively or predominantly humic of a dark color that also penetrates the underlying horizon.

RESULTS AND DISCUSSIONS

Phenological traits

Data on flowering phenology were recorded every two days during the flowering period. The beginning of flowering, the peak flowering period and the end of flowering were determined by subjective observations.

In addition to subjective observations, measured data were also recorded, based on the analysis of 30 flowers and fruits from each cultivar. The samples required for the analyses were collected randomly, collecting no more than one sample from each inflorescence or shoot. Flowering times were determined for all 12 old apple cultivars.

Table 2. Flowering time groups and varieties distribution

Flowering period	Variety
Very early	Gravenstein Papirovka
Early	Renet de Landsberg
Medium	Bănățesc Belle Fleur Jaune
Late	Domnesc Belle de Boskoop Renet de Canada London Pepping Parmen Auriu Jonathan
Very late	Trauben

According to data registered in the orchard the flowering times were divided into five flowering time groups on the basis of the beginning of flowering period. The groups are: very early, early, medium, late, and very late (Table 2).

The fruit traits linked with phenology are the times of harvest and eating maturity. As required by the UPOV guidelines (2005), the harvest maturity was scored using five categories and the eating maturity using nine, ranging from very early to very late.

Morphological traits

In Table 3 there were recorded data relating to the average morphological traits and their state of expression, according to UPOV (2005) guidelines'.

Table 3. Average morphological traits and their state of expression

Variable	Variety											
	T	**B**	**D**	**BFJ**	**BB**	**RC**	**LP**	**PA**	**RL**	**JO**	**G**	**P**
Average fruit weigh (g)	94.8	73.3	170.3	152.1	140.2	116.9	109.8	112.0	119.1	111.1	139.8	84.3
Average fruit heigh (mm)	62.2	57.2	76.9	74.4	56.8	57.8	56.9	57.2	54.9	56.0	64.3	58.4
Average fruit diameter (mm)	70.3	59.0	83.8	69.2	73.2	65.5	68.3	64.3	68.1	67.1	72.8	62.5
Fruit shape	3.1	4	4.2	2.1	4.1	4.2	3	3.3	3.1	2	3.3	2
Ground colour	2	2	4	2	3	3	4	2	2	3	2	2
Over colou	1	2	3	1	3	1	5	1	1	3	1	1
Presence o stripes	1	2	3	1	1	1	1	4	1	3	3	1
Colour of flesh	2	4	2	2	3	3	2	3	2	2	2	1
Taste	2	2	2	2	1	2	2	2	2	2	2	2
Flesh firmess	4	3	4	2	4	4	4	3	3	4	4	3

The analyses were performed between 2019 and 2022, and observations were made on each cultivar. It is important not to average the categories determined over a number of years; the category characteristic of each individual variety is taken as that which occurs most frequently in the separate annual classifications. Figure 2 shows fruits exterior characteristics (fruit size and shape, colour and presence of stripes).

Analyzing the results for these old apple varieties, we can say that fruit genetic traits are very important in today's breeding programmes for new cultivars, like fruit weight, shape, skin colour and flesh taste, stand out in explaining the variability (Ficzek, G. et al., 2017; Urrestarazu, J. et al., 2017).

Therefore, the conclusion that could be made is that these apple varieties present a good gene pool for these traits in breeding programs for new competitive commercial apple varieties. The above mentioned characteristics are defined by the market and consumers and play a major role in future breeding programmes.

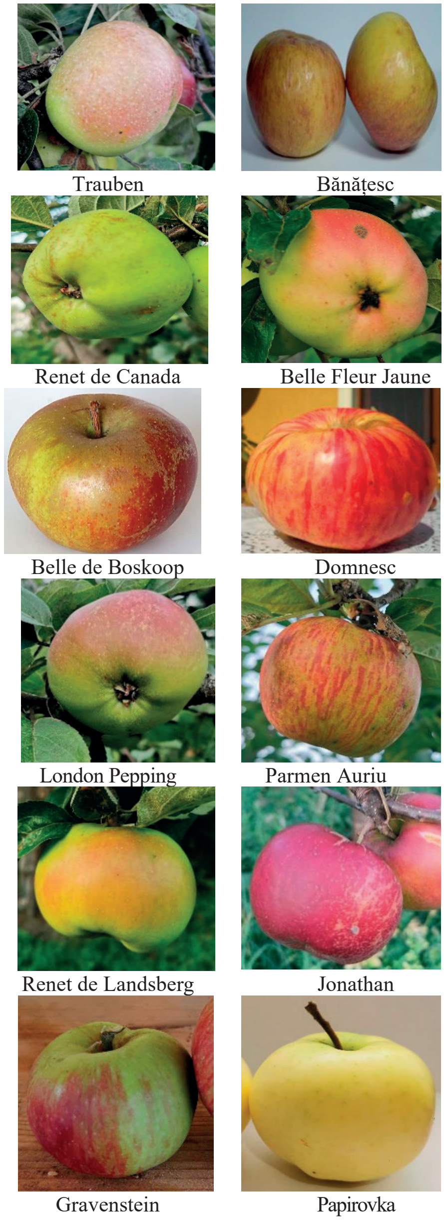

Figure 2. Fruits exterior characteristics

In addition to phenological and morphological descriptions, it is also advisable to carry out a series of genotypic analysis. The determination of the genetic fingerprints of the cultivars is of importance not only for the identification of gene bank accessions, but also to check the trueness-to-type of reproductive materials in nurseries. Well-chosen genetic markers can also be used to detect specific characteristics related to resistance, fertilisation, growth and fruit quality parameters (Hemmat M.S.K.B. et al., 2003; Kiprijanovski M. et al., 2020).

Lespinasse, since 2009 consider that a new European organization must be promoted to develop pre-competitive research on pome fruit genomics and molecular breeding, aiming finding solutions to the general and specific needs of the different pedo-climatic conditions of the European basins of production.

CONCLUSIONS

According to results of this research, it can be concluded that investigated apple varieties, are valuable sources of desirable genetic characteristics including important morphological and nutritional characteristics of the fruits.

The material presents valuable growing, yielding and fruit quality characteristics which make them valuable and useful in many aspects.

The apple cultivars were divided into five flowering time groups on the basis of the beginning of flowering period: very early, early, medium, late, and very late.

The results will contribute to the long-term conservation and sustainability in the cultivation of apple varieties, as well as to promote the value of conservation and the importance in the use of germplasm sources in apple.

Additional genetic fingerprinting of apple cultivars is needed. Until such work is undertaken, these genotypes should be conserved and analyzed for useful traits.

The results on this direction of research could be used in order to answer the new challenges concerning the drastic reduction of pesticides, the consequences of the climate changes and the need for better fruit quality and food safety.

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THE INFLUENCE OF MACERATION TECHNIQUES ON THE POLYPHENOLIC CONTENT OF THE WINES FROM NEGRU AROMAT GRAPE VARIETY

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Abstract

The aim of the present study was to establish the influence of maceration techniques on the polyphenol and aroma compounds of wines obtained from Negru aromat grapes, a variety created at the Research and Development Institute for Viticulture and Enology Valea Calugareasca, Romania. Three maceration techniques were applied as follows: submerged cap, pump-over (must recirculation) and delestage (several rack and return processes). Wines were evaluated and compared by chemical and sensory analyses, in order to determine which one is most suitable for this grape variety and can lead to well-balanced wines. With higher amounts of anthocyanins (377 mg/L), total polyphenols (3478 mg/L) and a more intense aroma of fresh fruits (cherries, bitter cherries, black currants, grapefruit), the wines obtained through pump-over technique applied during the phase of maceration-fermentation were identified as being of optimal quality. Thus, must pump-over is recommended for the production of Negru aromat wines, but more studies are warranted to optimize the winemaking process.

Key words*: Negru aromat, wine, polyphenols, anthocyanins, maceration-fermentation techniques*

INTRODUCTION

In wines, in addition to clarity, other important parameters for quality evaluation are represented by colour and aroma. These two characteristics are especially important for the wines obtained from Negru aromat, which is a red aromatic variety. To enhance both of these parameters, in wine technology a process of skin maceration is applied, in order to extract the main colour and aroma compounds. For this reason, red wines, as well as aromatic wines are produced with maceration and researchers have always tried to improve these processes (Puertas et al., 2008; Şener and Yıldırım, 2013; Cojocaru and Antoce, 2017; Tartian et al., 2017, Cerbu et al. 2021). The maceration process is one of the most important factors influencing the quality of red wines, as it contributes not only to the colour but also to the structure through the extracted tannin levels (Yacco et al., 2016). Certain technological steps and treatments applied during winemaking can influence the content of phenolic compounds extracted from grapes, with direct effect on colour and taste (Vrhovsek et al., 2002; Cojocaru et Antoce, 2017). The skins and seeds have a significant role in red grape vinification because they are the main source of phenolic compounds which are extracted into wine during the maceration-fermentation process of grape marc (Cejudo-Bastante et al., 2011; Zamfir et al., 2008; Zamfir et al., 2012). Phenolic compounds (anthocyanins, phenolic acids, procyanidins, etc.) accumulate in the solid parts of the grapes (bunches, peel, seeds), and their content is variable depending on the variety, the level of grape ripeness, the climatic conditions and, last but not least, the winemaking technology applied (Baiano et al., 2016; Rodríguez-Delgado, 2002).

MATERIALS AND METHODS

The current study was carried out at the Research and Development Institute for Viticulture and Enology Valea Calugareasca, Dealu Mare vineyard, during 2020-2021, by using grapes of the Negru aromat variety as raw-material.

A quantity of 150 kg of grapes were harvested in September 2020, at phenolic maturity.

The harvested grapes were divided in three batches for micro vinification experiments with the purpose to identify whether the application of a specific maceration-fermentation technique leads to statistically significant differences in wine composition and/or organoleptically observable treats.

The experimental variants, which differ only in the maceration-fermentation method, were coded as follows:

V1 - submerged cap (classical technology) is the control variant, where the cap is periodically crushed and mixed with the must, to facilitate the tannins extraction from the seeds;

V2 - pump-over (must recirculation) is the variant with recirculation, in which the must from under the cap is periodically pumped above the cap to facilitate the tannins extraction;

V3 - delestage (several rack and return processes) is the variant in which the must is completely separated from the cap and the cap is crushed for oxygenation.

The submerged cap technique was applied three times per day (morning, noon, evening), the pump-over was twice per day (morning, evening), and the Dele stage was performed in the first and last day of the process.

Each experimental variant consisted of 47 L of grape mash resulted after crushing and destemming of grapes. The crushed grapes in each variant were inoculated with selected yeasts *Saccharomyces cerevisiae* Viniferm TTA (Agrovin, Spain) in a concentration of 9 g/variant and treated with proteolytic enzymes Lafase fruit (Laffort, France) in a concentration of 1.5 g/variant in order to facilitate the extraction of red wine colour.

The maceration-fermentation process of the must was carried out at temperatures between 23-28°C, for 6 days, and when density reached 1000 g/cm^3, the batches were pressed using a Hydropress TH 40 L (Inderst, Italy).

The following analyses were performed during the maceration process: sugar content (refractometric method, OIV 2021a); total acidity (titrimetric method, OIV 2021b); pH (potentiometric method, OIV 2011); anthocyanins and total polyphenols (OIV, 2021c; Cayla and Renard, 2008) colour intensity and hue (spectrophotometric method, OIV 2021c).

Three types of wines were obtained and used to compare the influence of maceration technique on phenolic composition. The resulted wines were treated with 65 mg/L of sulphur dioxide for antioxidant protection and left for maturation in 25 L glass demijohns.

Wine samples were analysed after 6 months, determining the following parameters: determination of anthocyanins by the Ribéreau–Gayon-Stonestreet method was calculated with the formula Anthocyanins (mg/L)=875*Δd (Ribéreau-Gayon et Stonestreet, 1965); total polyphenols index by the Folin Ciocâlteu method was calculated with the formula Polyphenols (mg GAE/L)= Dilution*(A750-b)/a (OIV, 2021d); tannins were calculated with the formula Tannins (g/L) = Dilution*(0.0025+0.3647*DO550) using the leucoanthocyanidin method (Ribéreau-Gayon et Stonestreet, 1966). The colour analyses of the wines were performed using the CIELab method (Ribéreau-Gayon et al., 2006; OIV, 2021e).

The organoleptic profile of the wines was evaluated by a panel of wine experts by using specially designed tasting sheets (Antoce and Nămoloşanu, 2007).

RESULTS AND DISCUSSIONS

At harvest, the grapes of Negru aromat had good maturation parameters, such as sugar content of 236.4 g/L, total acidity of 3 g/L tartaric acid (39.4 milliequivalents/L), a gluco-acidmetric index of 78.8. The 100 berries weighted 144.89 g and the level of grape spoilage was under 5%. The berry composition index, that is the ratio of the liquid and solid fraction of berries, reached a value of 5.29.

Evolution of phenolic composition during the maceration-fermentation process

Throughout the maceration-fermentation process the anthocyanins and total polyphenols registered a continuous increase until the moment when the wine was separated from the pomace.

The highest amount of anthocyanins was recorded in the variant produced with must pump-over (V2), reaching 426 mg/L on the sixth day of maceration (Figure 1). Regarding the total polyphenols content, from Figure 2 it can be observed that the must pump-over variant (V2) registered values close to the control wine (4256 mg/L versus 4145 mg/L), while for the delestage variant (V3), a much smaller amount of polyphenols (2367 mg/L) was extracted.

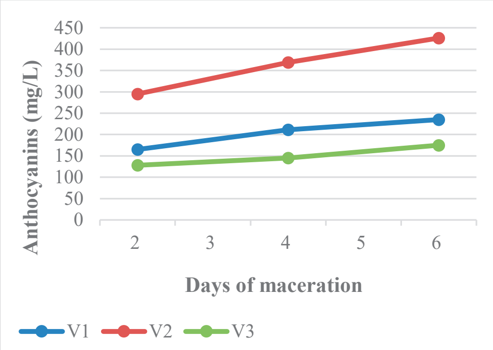

Figure 1. Evolution of anthocyanins during the maceration-fermentation process

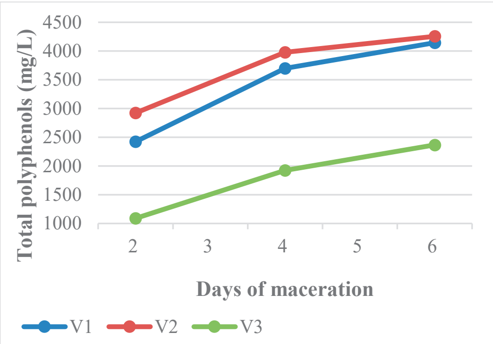

Figure 2. Evolution of total polyphenols in during the maceration-fermentation process

Colour intensity and hue showed a continuous increase during the maceration-fermentation process in all experimental variants (data not included for brevity).
The colour intensity values are quite close in all variants, but the wines obtained by pump-over (V2) had the highest colour intensity, and the wines obtained by delestage (V3) had the lowest colour intensity. Regarding the hue of the Negru aromat wines, the highest value was registered by the delestage variant (0.49), and the lowest value was registered by the submerged cap variant (0.439), showing that oxidation effect was higher in the delestage procedure.

Evaluation of wine quality

Red wines were evaluated and compared by chemical and sensory analyses after 6 months.
The alcohol content of the wines, irrespective of the maceration technology, was around 14.5% v./v., a concentration specific for the region and determined by the high amount of sugar in grapes at harvest.
The fermentation process went well in parallel with the maceration, most of the sugar being transformed in ethanol. Some differences were however observed, the control wine (V1) ending up as a dry wine with a 3.56 g/L of sugar, while the other variants remaining as semi-dry wines with 4.81 g/L (V2) and 7.93 g/L (V3) sugar, respectively.
The titratable acidity and pH affect the wines and other beverages stability and colour (Meng et al., 2012; Ju et al., 2021). The titratable acidity of the wine samples varied in the normal range for red wines, from 3.75 g/L to 4.35 g/L. The wine obtained by submerged cap (V1) had the highest titratable acidity content, reaching 4.35 g/L, followed by the wine obtained by must pump-over (V2) with 4.20 g/L.
As regards the pH, considering that recent studies recommend values between 3.0 and 4.0 for most wines to ensure balanced sensory properties and good stability (Forino et al., 2020), it was observed that Negru aromat placed at the upper end or higher of the optimum range. The pH values of the Negru aromat wines were 3.99 (V3), 4.06 (V1) and 4.16 (V2), respectively.
The results presented in Figure 3 show the influence of the extraction methods on the final content of total polyphenols, anthocyanins and tannins in Negru aromat wines. It can be seen that, the maceration done by must pump-over (V2) favoured an increase of anthocyanins content with 58.63% and a total polyphenol increase with 17.52% compared to control wines (V1), while the delestage method lead to lower values compared to both other technologies. The level of tannins in grapes and wines is influenced by the maceration-fermentation technique used (Figure 3), being higher in the wines obtained by delestage (1608 mg/L), compared to the other two variants (1152 mg/L

for the submerged cap, respectively 1403 mg/L for the must pump-over).

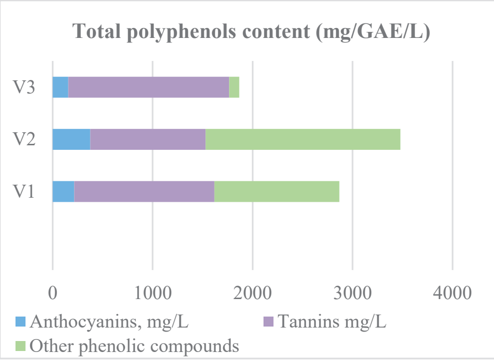

Figure 3. The influence of the maceration-fermentation method on the content of total polyphenols, anthocyanins and tannins in wines

The results regarding the colour of Negru aromat wines were obtained with the CIElab system. In this system, the colour can be decomposed into three independent orthogonal parameters L*, a*, and b*. Parameter L* indicates the lightness (or darkness) of the colour, while parameters a* and b* indicate the components of the hue (OIV, 2021). By analyzing these CIElab parameters we can numerically describe the colour characteristics of wines (Li et al., 2017; Ju et al., 2021).
Among the analyzed samples (Table 1), the colour of the wine obtained by delestage was the brightest, having the highest L* value (23.66), and the wine obtained by must pump-over was the darkest, having the lowest L* value (20.09). With these L values it is evident that the wine sample clarification process was not completed at the date of measurement.

Table 1. The CIELab colour parameters of the wine samples produced with various maceration technologies

Variants	Colour parametres				
	L^*	a^*	b^*	H^*	ΔE^*
V1	20.39	46.65	36.56	38.09	14.39
V2	20.09	46.80	37.71	38.86	14.46
V3	23.66	49.15	32.87	33.78	14.54

Parameter a* represents the position in the colour space between the red and green colour components, with values under 0 for green and over 0 for red, and parameter b* represents the position in the colour space between the blue and yellow components of colour, with values under 0 for blue and over 0 for yellow (Ju et al., 2021). Therefore, it can be observed that in the space a* and b* we have only positive values (Figure 4), meaning that the wines have a colour composed of a mixture of red (parameter a*) and yellow (parameter b*). Wines obtained by submerged cap and must pump-over are very similar in colour, while wines obtained by delestage show a slight shift towards more red and less yellow, which may indicate that due to a higher level of tannins there is a higher polymerisation of polyphenols and stabilisation of anthocyanins for the later winemaking method.

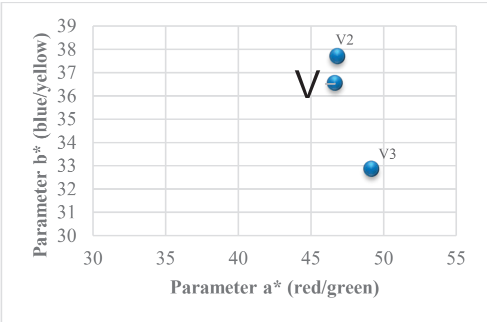

Figure 4. Variation of colour in Negru aromat wines obtained by three maceration techniques

The colour difference ΔE* is determined by the contribution of the three parameters L*, a* and b*, is calculated with the formula $\Delta E^* = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ and characterizes the general colour difference between wine samples (Ju et al., 2021; Perez el al., 2007). As shown in Table 1, the colour difference (ΔE*) between the wine samples is relatively small.
It was observed that the maceration-fermentation techniques by submerged cap (V1) and must pump-over (V2) lead to wines with a lesser red colour component, while delestage (V3) considerably improved the redness of wine, fact that most likely makes this type of wine more appealing to the consumers.

Sensory evaluation of wines

The sensory evaluation of the wines consisted of the visual, olfactory and taste examination.
In the organoleptic profile resulted from the quantitative sensory analysis and depicted in figure 5, it can be observed that the wines obtained by the must pump-over (V2) and the ones resulted after using the submerged cap

technique (V1) are the most balanced in all aspects. These wines have less bitter and less astringent tannins compared to the wines obtained by delestage (V3), which are perceived as rough, even though are better structures, a fact also proven in the analytical analysis by the higher content of tannins. The wine obtained by delestage (V3), from a taste point of view, presented a polyphenolic imbalance.

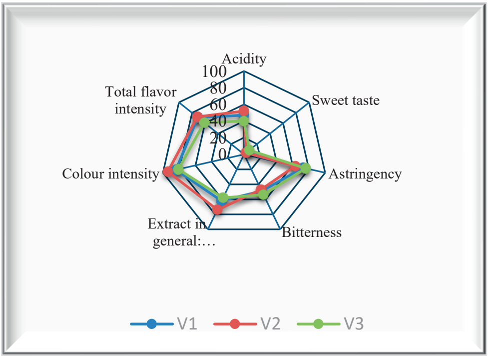

Figure 5. The influence of maceration-fermentation methods on the organoleptic profile of wines

As shown in figure 6, the wines obtained by the pump-over method (V2) differ from the other two wine samples by a more intense aroma of fresh fruit (cherries, bitter cherries, black currants and grapefruit). The control (V1) stands out with slight vegetal notes (fresh grass), and the wine obtained through delestage (V3) shows slight notes of spices (pepper, bitter chocolate) and especially vanilla.

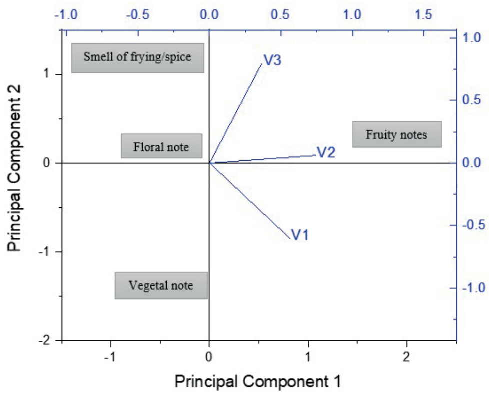

Figure 6. The influence of maceration-fermentation methods on the aromas of wines

CONCLUSIONS

This research confirms that the use of different maceration-fermentation techniques induces certain changes in the content of total polyphenols, anthocyanins, colour and on the sensory characteristics of red wines.
The anthocyanin and total polyphenols content in wine is clearly dependent on the maceration technique used. The results obtained for anthocyanins vary from 156 mg/L when the maceration was carried out by delestage (V3), to up to 377 mg/L when the maceration was carried out by must pump-over (V2).
The results show that the total polyphenols extraction ranged from 1867 mg/GAE/L when wine was obtained by delestage (V3), to 3478 mg/GAE/L when must pump-over method was applied (V2).
Regarding the content of tannins in the Negru aromat wines, it was observed that the use of delestage maceration technique (V3) was correlated with an increase in the level of this compound.
The analyses regarding the colour, it was found that the wines obtained by the submerged cap (V1) and the must pump-over (V2) had a very similar colour (red), while the wines obtained by delestage (V3) showed a slight shift towards a more red and less yellow.
The wines obtained through the maceration-fermentation technique by the must pump-over (V2) differ from the other two wine samples by a more intense aroma of fresh fruit (cherries, bitter cherries, black currants and grapefruit).
All things considered, the maceration by pump-over technique is recommended for the production of Negru aromat wines to be consumed within a few years from production, as it leads to well-balanced, full-bodied, intensely coloured and with specific fruity aroma, while delestage (V3) may be a better option for the case of wines meant for aging.

ACKNOWLEDGEMENTS

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FERMENTATION TEMPERATURE AND DURATION EFFECT ON THE QUALITY AND ANTHOCYANIN CONTENT OF KADARKA WINE PRODUCED IN THE MINIȘ (MÉNES) WINE REGION

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Abstract

Grape growing and winemaking has a very long history around the world, where the geographical conditions and the climate is favourable. Romania could be listed among these regions, where can be found numerous red and white wine regions. Miniș region is famous for one of its red wines made from a traditional variety named Kadarka. The aim of the present experiment was to examine the quantitative and qualitative parameters of the wine made from Kadarka obtained under different conditions undertaken during fermentation on skins , and to knowledge how these parameters change with the different fermentation temperatures and duration and also measuring the anthocyanin content of the wine. The analyses quantitative and qualitative parameters were not changed under different tested conditions such as maceration time or temperature. However, the results showed that higher temperature (28°C) and shorter maceration period (12 days) gives a maximum anthocyanin content of 380 mg/L. The sensory evaluation of the resulted wines, revealed that a shorter maceration period and a lower temperature during fermentation on skins ensure better results for Kadarka variety.

Key words*: fermentation, Kadarka, Miniș, wine.*

INTRODUCTION

Winegrowing and winemaking have a very long history that it is cultivated back to 8000 years. This is one of the oldest plants grown by mankind. Grape and wine have great cult significance in many religions. Winemaking hides its beauty in complexity (Antoce & Stockley, 2019).

The geographical location, favourable climate and soil properties for grape growing, Romania plays a significant role in European wine production. The favourable climatic conditions, especially the rainfall and temperature during the growing season, benefits the production of quality wine. On the south-facing slopes of Romania's wine-growing regions, the grapes will ripen properly while their sugar and acidity will be balanced. Some "foreign" grape varieties show better development in Romania than in their native regions. For example, Muscat Ottonel is a grape variety bred in France, but in the Târnave wine region has an excellent taste, aroma, and develop in its wine (Balla, 2003; Csomós, 2003).

Based on data from the International Organization of Vine and Wine (O.I.V., 2007), the grape-growing area of Romania shows an increasing trend from 1986 to 2000 and a decreasing trend between 2001 and 2007. The areas changed as follows according to the past years: 243,000 ha between 1986–1990, 251,000 ha between 1991 and 1995, 252,000 ha between 1996 and 2000, 233,000 ha between 2001 and 2005, 213,000 ha in 2006 and 209,000 ha in 2007. This average area size accounts for approximately 1.7% of Romania's agricultural areas (O.I.V., 2007).

According to new research, a regulated and moderate consumption of wine has a positive effect on health (German & Walzem, 2000). This is due, among other things, to the high anthocyanin content of red wines (Garcia-Alonso et al., 2009).

Anthocyanin is an important quality parameter that contributes to antioxidant activity and gives an appealing colour to red grapes and wine (Mateus et al., 2002; Cliff et al., 2007; Pereira de Freitas et al., 2017). An anthocyanin-rich diet may prevent inflammation and also protect against heart diseases (He et al., 2012; Yoo et al., 2013). A young red wine anthocyanin content can have an average of 500 mg/l, making them one of the important bioactive compounds in wine (Wu et al., 2006). Anthocyanins can also help in the prevention of colon cancer and colorectal cancer (Cooke et al., 2006; Jing et al., 2008; Somerset & Johannot, 2008), coronary heart diseases, and non-alcoholic liver diseases (Gronbaek et al., 2000; Newcomb et al., 1993; Anderson et al., 2005; Dunn & Schwimmer, 2008). One of the grape varieties with high anthocyanin content is Kadarka (Csávossy, 2003; González-Neves et al., 2012).

The origin of Kadarka is unclear, it is probably originating from Asia Minor, the convar. *pontica*, subconvar *balcanica*, provar. *mesocarp*, subprovar. belongs to the dalmatica group. South Slavs (Serbs, Ráks) brought it to the Miniș wine region while fleeing from the Turks. On the hillsides of the Mocrea settlement, "Turkish grappe" was grown B.C. a red wine grape variety, also called Negru moale in Romanian. In the middle of the 19th century, in Miniș and later in Gyorok, aszú was made from the shrunk berries of Kadarka. By 1872, it was one of the most widespread grape varieties in the wine region (Csávossy, 2003; González-Neves et al., 2012).

It is a less frost-resistant variety, it tolerates drought relatively well, and the vine grows strongly even in sand, the canes are thick, brownish-grey, striped, and have short internodes. The small, cobwebbed buds develop erect canes, why it is also suitable for training without support, requires short pruning. It does not require intense canopy management. Its shoot trip is characterized by a bronze-yellow-green colour and woolliness (Prohászka, 1986).

Leaves are medium-sized or large, shiny, dark green, thick-textured, and rough to the touch. Leaf blade 3-5 lobed, wavy with a woolly backside.

The cluster is cylindrical, dense, and medium-sized, prefers to be close to the ground.

The fruits are medium-sized, round, blue-black when fully ripe, thin-skinned, juicy, and characterized by a pleasant taste. It is late-ripening, fruits ripen in August, but it reaches technical maturity in mid-October. Sunlight requirement is high. It is usually abundant, but its productivity is strongly influenced by the conditions of fertility (Bényei et al., 1999).

It shows no resistance against diseases, especially in rainy seasons it is heavily damaged by fungal diseases. Because of its weak resistance against rot, it is often harvested already in September. In a good location and a favourable year, can reach 18-20°Bx must degrees.

The wine has a characteristically spicy aroma and flavour, is rich in tannic acid, has a ruby red colour, is thinner and fresher on looser soil, fuller-bodied on firmer soil, and richer in colourants, the latter being a characteristic of Minişi Kadarka (Cliff et al., 2007).

It is a component of "Miniș red", initially it was used at a rate of 70%, and currently 20-30% is added (Ştefan et al., 2017).

The aim of the experiment was to determine the influence of different fermentation temperatures and durations on the anthocyanin content and quality of the resulting Kadarka wines when using the technology of red wine production with fermentation on skins.

MATERIALS AND METHODS

The winemaking was carried out 26 km from Arad at Păuliș, Wine Princess S.R.L. The vineyard occupies an area of approximatively 70 ha. One of the most important varieties is Kadarka. The harvest began on 20th September, which ended on 17th March. The mash was pumped into the fermentation tanks. In each tank 7,990 L of mash was placed, leaving 20% fermentation space. Sulphite addition to the mash took place depending on the health of the grapes. In this case, were treated with 50 mg/L SO_2. The mash was cold-soaked for 48 hours at 9°C after was circulated with a pump three times a day in order to dissolve the colour. To start the fermentation and enzymatic treatment, the temperature was raised to 15°C. 23.97 g of Pectosol enzyme suspension per container was dissolved in 2 L of 20°C water and mixed with the mash. The fermentation temperature was controlled by computer for each fermentation tank.

During the winemaking, 17,000 kg of Kadarka grapes were processed. After removing the

stems and crushing the berries, 15,980 L of mash was fermented.
Two batches were separated K1 and K2. K1 was fermented at 25°C for 8 days, while K2 was fermented at 28°C for 12 days. Each batch was fermented in 10,000 L stainless steel wine tanks equipped with cooling dimpled jacket, and 20% of the tank was left empty for the fermentation. The fermentation of K1 was completed on 15th October and K2 on 19th October.
After the fermentation was finished the sugar content was measured and then the free-run wine was separated from the marc, then it was pressed at a maximum of 1.5 bar. The press wine and the free-run wine were then blended at a proportion of 80/20, and 11,900 L of wine was produced.
On 20-21th of December, when the malolactic fermentation ended, the first organoleptic examination was carried out and the basic laboratory tests were made (alcohol, titratable acid, volatile acid, free sulphur content, pH level and anthocyanin content).
The new wine was put into barrique barrels. The wine was aged for three months, after a second organoleptic examination and laboratory tests were made.
For the evaluation, we used a 100-point rating system issued by the International Organisation of Vine and Wine (O.I.V.). Besides the colour, clarity, intensity, aroma, bouquet and taste the overall impression and harmony were rated.

RESULTS AND DISCUSSIONS

The result of the qualitative examination of Kadarka grapes before processing was 225.32 g/L sugar content, while the titratable acid content was 7.3 g/L expressed in tartaric acid. The wine's sugar content was measured 8 days after the fermentation stopped, the results were between 3.9-4.0 g/L, since both variants were fermented as a dry wine.
The effect of fermentation temperature and maceration period on Kadarka wines alcohol content is presented in Figure 1.
There is a difference of 0.2 volume % between the variants fermented for 8 days and 25°C and the variants fermented for 12 days and 28°C (Figure 1). The K1 has an alcohol content of 13% by volume and K2 had an alcohol content of 13.2% by volume on 20th of December. It can be determined that the results measured on 15th March, after three months of maturation, did not show any deviation either, the measured alcohol content did not change in the case of both variants.

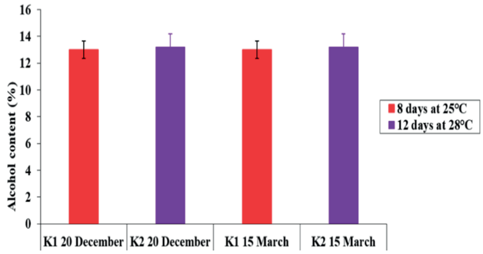

Figure 1. Changes in alcohol content on different temperatures in K1 and K2 inox tanks

The effect of fermentation temperature and maceration period on Kadarka wines titratable acidity is presented in Figure 2.
The titratable acidity of the wine fermented for 8 days at 25°C is 6.2 g/L, while the titratable acidity of the wine fermented for 12 days at 28°C is 6.4 g/L, which was measured on 20th of December. The analysis on 15th March recorded the following titratable acidity results: K1 6 g/L and K2 6.1 g/L. After three months of maturation there is a difference of 0.1 g/L between the two tanks. The variant fermented for a longer time and at a higher temperature had higher titratable acid content in both tests. At the same time, a decrease in acidity can be observed in the case of both variants during maturation, which was caused by the precipitation of tartaric acid during malolactic fermentation.

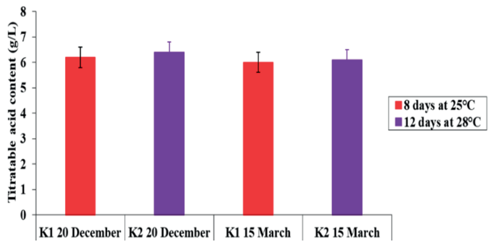

Figure 2. Changes in titratable acid content in wines fermented at different temperatures in K1 and K2 inox tanks

The effect of changes in fermentation temperature and duration on the volatile acid content of Kadarka

Under our experimental conditions the results of the volatile acid content differ slightly between the K1 and K2 (Figure 3). The first laboratory test gave the following results: the volatile acid content of wine fermented for 8 days at 25°C was 0.52 g/L, and the volatile acid content of wine fermented for 12 days at 28°C was 0.61 g/L. The results of March: K1 0.54 g/L, the K2 0.63 g/L, there is also a difference. The results show that the volatile acid content of both variants increased by 0.02 g/L during the three-month maturation period.

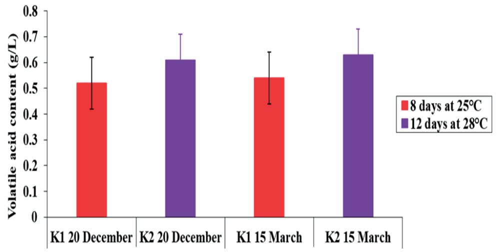

Figure 3. Changes in volatile acidity in wines fermented at different temperatures in K1 and K2 inox tanks.

The effect of fermentation temperature and maceration period on Kadarka wines on free sulphur dioxide is presented in Figure 4.

During the analysis on 20th of December, 16 mg/L sulphur dioxide content was measured for K1 and 21 mg/L for the K2 variant. In the laboratory test on 15th March 21 mg/L was measured from the wine fermented for 8 days at 25 °C and 19 mg/L of the K2 fermented for 12 days at 28 °C (Figure 4).

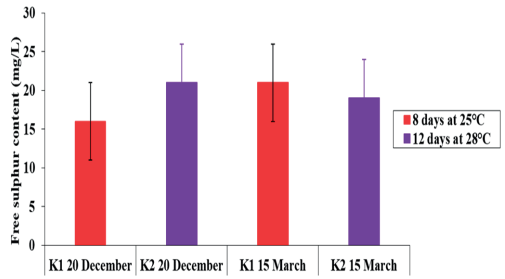

Figure 4. Changes in free sulphur dioxide in wines fermented at different temperatures in K1 and K2 inox tanks

The effect of fermentation temperature and maceration period on Kadarka wines on pH is presented in Figure 5.

The Kadarka pH content values were between 3.0 and 3.1 (Figure 5). The pH content of K1 was 3.1 and K2, 3 at the time of the first laboratory test. During the March test, the pH content showed the same results as the first time. So, during the three-month storage of Kadarka, the pH content of the samples did not change.

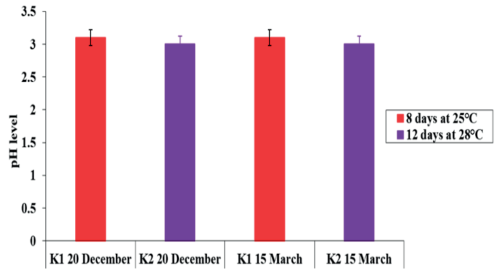

Figure 5. Changes in pH level in wines fermented at different temperatures in K1 and K2 inox tanks

The effect of fermentation temperature and maceration period on Kadarka wines on anthocyanin content is presented in Figure 6.

It is clear that there is a difference between the K1 and K2 variant in terms of the anthocyanin content (Figure 6), one of the significant factors determining the colour of red wines. The anthocyanin content of the wine fermented for 8 days at 25°C was 345 mg/L, while the anthocyanin content of the wine fermented for 12 days at 28°C was 380 mg/L after malolactic fermentation. The March results were as follows: K1 was 320 mg/L and K2 was 357 mg/L. Although the anthocyanin content of the variant fermented at a higher temperature and for a longer time was higher, it showed a decrease of 23 mg/L during maturation. The decrease can also be attributed to the anthocyanin content of K1. The formation of the anthocyanin tannin complexes during wine maturation decrease the free anthocyanin concentration.

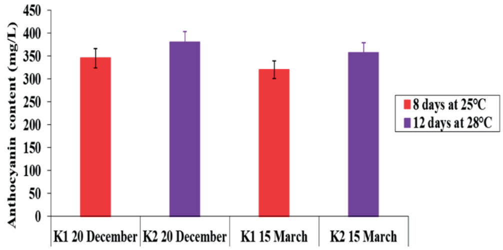

Figure 6. Changes in anthocyanin content in wines fermented at different temperatures in K1 and K2 inox tanks

The results of the organoleptic tests of Kadarka
Examining the results of the first sensory test (Figure 7), the K1, fermented for 8 days and at 25°C, received a higher evaluation. The K1 variant scored lower than K2 for the colour and clarity parameters, which was also associated with lower anthocyanin content. However, the aroma of the K2 variant was not favoured by the longer maturation time (12 days) or the higher temperature (28°C), so its evaluation was lower than the K1. There is only a difference of 1 point between the taste and general impression in the case of the two variants. The total scores of the evaluation: K1 81 points and K2 80 points.

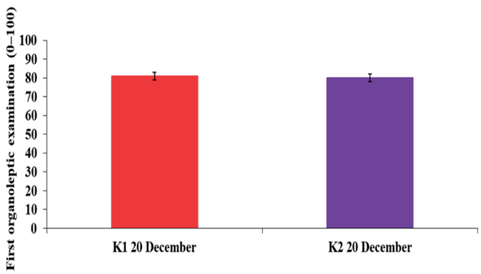

Figure 7. Scores of the first organoleptic examination obtained by wines fermented at different temperatures in K1 and K2 inox tanks

During the examination on 15th March, a difference of 2 total points can be seen between the variants, as in the first organoleptic examination (Figure 8). During barrel ageing, the intensity of the colours decreased, but the quality of the colours improved, which is also shown by the evaluation of the March test. In the case of both variants, the scores of the colour and clarity parameters increased. In addition to the aroma, the bouquet of wines appeared, and this parameter improved, but the K1 was considered better by the judges, it received 26 points for this parameter. Regarding the taste and general impression, the K2 developed less during the maturation, reaching the same average score as the K1, 45 points. The total score: K1 88 points and K2 variant 86 points. K1 variant fermented for a shorter time (8 days) and at a lower temperature (25°C) proved to be of better quality.

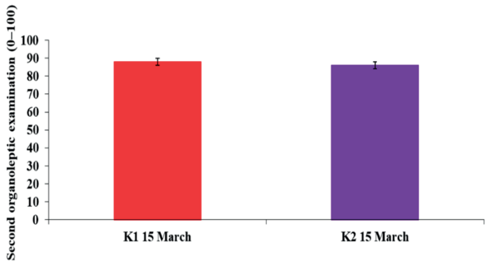

Figure 8. Scores of the second organoleptic examination obtained by wines fermented at different temperatures in K1 and K2 inox tanks

CONCLUSIONS

From the present study we can conclude that, the different temperatures and length of fermentation did not have a significant effect on the wine's alcohol, titratable acid, volatile acid, free sulphur content or pH level, but still these measurements were important to complete because they are the basic measurements regarding winemaking.

In terms of anthocyanin content, better results were achieved with a higher temperature and a longer fermentation period.

Regarding the organoleptic evaluation, K1 with a lower temperature and a shorter fermentation period scored higher (88 points). This grape variety genetically produces less anthocyanin, but is excellent and characteristic in its flavour and aroma substances, therefore, to preserve the aroma substances, fermentation at a lower temperature is recommended.

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MECHANISMS AND FACTORS INFLUENCING MCFA FORMATION BY YEASTS DURING GROWTH AND ALCOHOLIC FERMENTATION AND THEIR IMPORTANCE IN WINEMAKING

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Abstract

The medium chain saturated fatty acids (MCFA) are synthesized by the yeasts and released in fermentation media, where they contribute to fermentation aroma, but are also well known for their inhibitory effect on yeast growth. At low pH and in the presence of ethanol, only few mg/l of MCFA could arrest the AF. One of the mechanisms employed by the yeasts to protect themselves from the MCFA effects implies the conversion of the MCFA in ethyl esters, which are less toxic. In this way, fermentation aroma is even more enhanced, as ethyl esters of MCFA have more appealing sensorial qualities and are perceived at lower thresholds. Understanding MCFA's occurrence in grape must and wine and their inhibitory mechanisms can be useful for a better predictability and control during winemaking process. Certain technological factors were found to dramatically affect the concentrations of MCFA during winemaking, among which are the grapes ripening process, several technological interventions during winemaking and the yeast strains, all influencing style and perceived quality of wines. Furthermore, due to their inhibitory effects on yeasts, the MCFA have drawn the attention of researchers and producers as agents able to reduce the doses of SO_2 necessary for the cessation of alcoholic fermentation (AF) in order to obtain sweet wines. In this review inhibitory mechanisms are discussed, along with possible ways to control the MCFA concentrations during winemaking, also in the view of sweet wine production.

Key words: *MCFA, octanoic acid; decanoic acid; ethyl octanoate, ethyl decanoate, sweet.*

INTRODUCTION

Medium-chain fatty acids (MCFAs) are saturated fatty acids with C_6-C_{12} atoms which are volatile organic compounds produced in wines and other fermented beverages along with other major aroma constituents (Bardi et al., 1999; Zhao et al., 2017). Low to moderate concentrations of medium-chain fatty acids (MCFAs) and their ethyl esters (MCFAEEs) can contribute to positive aroma quality of wines and other fermented beverages (Borrull et al., 2015; Mina & Tsaltas, 2017). Accumulation of MCFAs during AF under certain conditions may inhibit growth of both yeast or lactic bacteria, which may lead to either an incomplete AF or an unachievable onset of MLF (Alexandre et al., 2004; Lonvaud-Funel et al., 1988). For those wines with high concentrations of MCFAs and problems of AF completion or MLF starting, the removal of these compounds must be taken into account (Lonvaud-Funel et al., 1985; Viegas et al., 1989). MCFAs are slightly soluble *in water, the solubility decreasing with the chain length. Their O: C ratio by mass is a simple way to point out the hydrophobicity increase, as the chain of aliphatic carbon increases. As the MCFAs hydrophobicity increases, their solubility in the phospholipids of yeasts membrane increases as well. The insertion of the undissociated acid forms inside membranes leads to an increase in their permeability and* consequently to a loss of their biological function (Viegas et al., 1989). Enhanced permeability of yeast cell membranes due to the presence of MCFAs during AF, promoted yeast cell death, especially under ethanol-induced proton influx, which determines the decrease of internal pH (Borrull et al., 2015; Leão & Van Uden, 1984; Viegas et al., 1989). Levels of ethanol concentrations to 12% or above, pH values lower than the MCFA's pK_a, as it happens in grape juice (3.0 to 4.0) and promoting those conditions which enable the biosynthesis of MCFAs in the fermenting medium, all lead to a decrease in *Saccharomyces cerevisiae* viability or cell death (Borrull et al., 2015; Viegas & Sá-Correia, 1997). In order to produce ATP through glycolysis, yeast cells need to maintain the internal pH around 6.0 to 6.5 during fermentation, thus, any factors able to lower the internal pH below 6.0 will stop the ATP production and therefore activate the autolysis enzymes, leading to a subsequent degradation of cell components (Alexandre & Guilloux-Benatier, 2006; Torello Pianale et al., 2022). Temperature of the fermenting media influences the inhibitory effect of MCFAs, for instance, a low temperature, as the one used for the white wine production, is able to reduce the loss of viability in *Saccharomyces cerevisiae*, while a higher temperature promotes higher cell death rates (Viegas & Sá-Correia, 1997). These factors and mechanisms are important for wine production, due to their consequences on the quality of the final product. Accumulation of MCFAs in the fermenting media during AF is an undesirable pathway of yeast metabolism, which increase the risk of sluggish or even stopping AF. Relatively recently, medium-chain fatty acids came in to the attention of researchers and producers of natural sweet wines at the international level as a means to reduce the doses of SO_2 used for stopping fermentation to produce sweet wines (Baniţă et al., 2023; Banita & Antoce, 2021; Baroň et al., 2017). The use of MCFAs for AF cessation is still under evaluation as an alternative method to reduce electric energy consumption necessary for cooling and the dose of sulfur dioxide required in the classical method (Baniţă et al., 2023; Baroň et al., 2017; Viegas & Sá-Correia, 1997). Moreover, the addition of MCFAs for AF cessation was found to reduce of the acetaldehyde content compared to the chilling process, as well as to reduce the diacetyl content compared to cross-flow filtration (Licek et al., 2020). Modulation of MCFA concentration during winemaking allows the oenologists to control the kinetics of AF to dryness or to stop the fermentation to produce wines with natural residual sugar.

MCFA PRODUCTION DURING WINEMAKING

MCFA production in wine is dependent on controllable *physical factors*, such as temperature or aeration during AF and less controllable *biological factors,* such as grape cultivar, grape microbiome or must composition, which includes nitrogen compounds, lipids and pH (Lonvaud-Funel et al., 1988; Rizk, 2016).

The effect of aeration regime - lipid biosynthesis and wine quality

Generally, yeasts need 4 to 8 mg/l O_2 at the end of growth phase in order to promote lipid biosynthesis, especially unsaturated fatty acids (UFAs) and ergosterol, which are known as "survival factors" (Andreasen & Stier, 1953, 1954; Bell & Henschke, 2005; Boll et al., 1980; Jean-Marie et al., 1996; Lafon-Lafourcade et al., 1979). These growth factors, UFAs and ergosterol, integrated into yeast cell membrane of *Saccharomyces cerevisiae* are produced only in the presence of oxygen and are necessary for yeasts to strengthen their resistance to stress factors such as extreme alcohol, temperatures, osmotic pressure (Costa & Moradas-Ferreira, 2001; Lafon-Lafourcade et al., 1979; Piper, 1995). In beer fermentation, yeast requirements for oxygen to promote sterol biosynthesis are between 0.1 to 0.3 mg O_2/gram of yeast (expressed as dry mass), while for UFA biosynthesis, a dose of 0.35 mg O_2/gram of yeast (expressed as dry mass), is considered sufficient for high gravity brewing fermentation (Kirsop, 1977; Rosenfeld et al., 2003). In the case of wine fermentation a larger dose of 0.5 mg O_2/gram of yeast (expressed as dry mass) is required for sterol biosynthesis, while for UFA synthesis the amount of oxygen is negligible (Salmon et al., 1998).

Overall, a more oxygenated fermenting media may be helpful in the case of nitrogen deficient grape musts, improving the use of proline nitrogen-use by yeasts by activating the responsible enzyme, namely proline oxidase (Bell & Henschke, 2005; Ingledew & Kunkee, 1985). The addition of nitrogen-containing

inorganic nutrients (diammonium phosphate) or even organic nutrients based on amino acids will activate and stimulate the multiplication of yeast cells in the beginning of AF phase, contributing to biomass increase, but without effect on yeast viability at the end phase of AF (Andreasen & Stier, 1953, 1954; Lafon-Lafourcade et al., 1979). The nitrogen is frequently a limiting factor for production of adequate biomass required to complete the fermentation and it is usually adjusted by winemakers, in accordance to the necessities of the selected yeast strain at the desired temperature during AF, following routine grape juice analyses for sugar concentration and yeast assimilable nitrogen (Bell & Henschke, 2005).
In order to influence yeast viability towards the end of AF, besides aeration, another solution is adding nutrients rich in "survivor factors" during rehydration of dry yeasts or during fermentation, as their reduced content affects physical characteristics of membrane, reducing the cell resistance to stress factors (Daum et al., 1998). Rehydration nutrients rich in sterols and UFAs will help completing AF by increasing the resistance of yeasts to stress factors (Lafon-Lafourcade *et al.*, 1979). The yeasts are able to directly incorporate into their membranes sterols and UFAs during growth and AF (Chen, 1980). The ergosterol plays a critical role in ethanol resistance in *Saccharomyces cerevisiae* (Ding et al., 2009) allowing for the increase of membrane rigidity, while high concentrations of ethanol can induce plasma membrane fluidity and consequently becoming toxic to membrane proteins, fact that stops cell growth and induce cell death (Jones & Greenfield, 1987). As the modern white and rose winemaking is often based on avoidance of the aeration and stimulation of production of acetate and ethyl esters, many winemakers use nutrients rich in "survivor factors" during rehydration of active dry yeasts, in order to incorporate ergosterol and UFAs inside yeast cell membranes.
Genes involved in acetate esters production, alcohol acetyltransferases *ATF1* and *ATF2*, are repressed by oxygen exposure during AF, while adding UFAs during AF represses only *ATF1*, while *ATF2* is overexpressed (Fujii et al., 1997; Peddie, 1990; Saerens et al., 2010; Yoshimoto et al., 1999). However, depriving the yeast of oxygen or creating hypoxic conditions during AF promote the formation and accumulation of MCFAs in wine, which decreases the viability of yeasts towards the end of FA and leads even to stopping FA (Borrull et al., 2015; Takashi, 1986).
Fatty acids are derived from degradation of storage and complex lipids, *de novo* synthesis and uptake from the environment (Klug & Daum, 2014). *De novo* lipogenesis starts in the cytosol and in mitochondria through acetyl-CoA-carboxylase encoded by cytoplasmic *ACC1* and of mitochondrial *HFA1* genes (Hasslacher et al., 1993; Hoja et al., 2004). After initiation of this process, elongation of the chain is based on the malonyl-CoA, the universal precursor of fatty acids, which serves as a two-carbon building block (Beld et al., 2015). Fatty acid elongation is generated by a FAS system of enzymes (Toke & Martin, 1996). Yeast contains two distinct fatty-acid synthases (FAS), the cytoplasmic FAS (*FAS1*), which is a large multifunctional enzyme producing over >95% of cellular fatty acids and, mitochondrial FAS (*FAS2*), a non-aggregated FAS enzymes similar to that of bacteria (Brody et al., 1997; Harington et al., 1993; Lynen et al., 1980; Schneider et al., 1997).
The MCFAs are by-products of lipid synthesis as a result of overexpression of fatty acid synthetase complex (*FAS1* and *FAS2*) activated by hypoxic conditions during AF and thus, being the main reason for which wines fermented in such conditions have increased concentrations (Restrepo et al., 2019; Saerens et al., 2010; Taylor & Kirsop, 1977). The increase of MCFA concentration in wine is thus a consequence of an active process under anaerobic conditions, as they are not needed for structural lipid synthesis (Bardi et al., 1999). As MCFAs are not necessary for cell function, MCFAs are released into the medium through passive diffusion across the cytoplasmic membrane and are not bound to cell structures (Bardi et al., 1999). The accumulation of MCFAs in fermentation media was found to be a result of the release from FAS complex of acyl-CoA, which is then hydrolysed in order to recycle CoA-SH, thus liberating the MCFA acyl part. Under these conditions, cell growth

stops, as the lipid biosynthesis becomes impossible (Bardi et al., 1999).
To counteract the anaerobic conditions and to improve yeast viability, $C_{16:1}$ fatty acid can be added in the medium, yeast cells showing higher viability and faster fermentation rates at 13°C. These cell membranes incorporated higher concentrations of $C_{16:1}$ and ergosterol, fatty acids with a shorter chain length and had a higher sterol/phospholipid ratio (Redón et al., 2009).
Controlling the oxidation reduction potential (ORP) during wine fermentation and changing it in the range of -100 to + 100 mV, revealed that the maximum concentration of MCFAEEs is produced in wines during AF when ORP is maintained at 0 mV (Xue et al., 2022). Controlled ORP at 0 mV during AF led to a higher flux of citric acid to cytoplasm, a moderate $NADP^+$/NADH ratio and highly expressed genes *ACC1*, *FAS1*, *FAA2* and *EEB1*, thus favouring the production of MCFAEEs, which therefore improve the aroma quality in wines (Xue et al., 2022).
However, overexpression of MCFA-ethyl ester synthases EEB1 and EHT1 is only slightly affected by oxygen exposure, MCFAEE production responding differently to MCFA precursors. Overexpression of *EEB1* gene responds well to the presence of octanoic acid and poor to decanoic acid, while the overexpression of *EHT1* gene responds to both acids, but still more to octanoic acid. Deletion of these genes decreases the MCFAEE production, confirming their involvement in ester production mechanisms. *EHT1* and *EEB1* genes may have both activities of synthesis and hydrolysis, because their overexpression did not enhance MCFAEE content, although are involved in MCFAs detoxification (Saerens *et al.*, 2006). A possible yeast detoxification metabolism involves ethyl esters biosynthesis through *EHT1* and *EEB1* genes and the role of *ATF2*, involved in acetylation of sterols, which is preferentially expressed under anaerobic conditions (Cauet et al., 1999; Saerens et al., 2010).
During the fermentation of high sugar musts, the accumulation of ethanol can increase excessively the production of reactive oxygen species (ROS), as a consequence of uncoupling electron transport chain from the ATPase, thus, being capable of damaging cellular constituents (Jones & Greenfield, 1987; Landolfo et al., 2008; Moradas-Ferreira *et al.*, 1996). The yeast cells hinder the ROS accumulation through modification of cellular lipid composition, triggering antioxidant defence mechanism such as GSH production. Any redox imbalance can result in oxidative stress (GSH depletion, lipid peroxidation, protein oxidation and, even worse, DNA damage) and can cause yeast cell death (Costa & Moradas-Ferreira, 2001; Piper, 1995). GSH and cysteine depletion under oxidative stress promotes the production of H_2S, a strong reducing agent known to be involved in the protection mechanism used by yeasts to detoxify ROS (Sohn & Kuriyama, 2001). The polyunsaturated fatty acids percentage plays an important role in oxidative stress resistance, which explains why in species better adapted against the oxidative stress, such as *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*, the proportion of these compounds is higher compared with that of *Saccharomyces cerevisiae* (Vázquez et al., 2019).

The effect of temperature regimen during AF

The temperature during AF for white/rosé wine production is generally lower than that for red wines. White and rosé wines need a temperature range between 15 to 18°C in order to develop and preserve the aromatic compounds and to control the yeast biomass during the process, while for red wine production temperatures over 24 to 28°C are more common, to allow for better phenolics extraction during maceration-fermentation phase. Lower temperature regimes may be implemented in case of fruity red wine production. The AF temperature is known to affect the concentrations of MCFAs and MCFAEEs in the final product. The fermentation of several grape white varieties (Feteasca alba, Pinot Gris and Chardonnay) using *Saccharomyces cerevisiae* strain Vinoferm Crio at different temperatures showed the effect of temperature on MCFAs and MCFAEEs production (Csutoras et al., 2022). The controlled temperatures between 15 to 16°C during AF lead to a lower concentration of these compounds, while

elevated fermenting temperatures of 25 to 26°C, lead to a higher concentration of these compounds in the resulted wines (Csutoras et al., 2022).
Compared to white wines, generally, red winemaking requires higher temperatures during AF for the skin maceration and, as a consequence, increased MCFA and MCFAEE concentrations are also produced in the final wines. This happens even more if there is no aeration during pump-over.
Several experimental variants carried out on Carménère grape variety macerated-fermented at different temperatures (24, 28 and 32°C) and inoculated with *Saccharomyces cerevisiae* strain EC1118 revealed that increasing temperature from 24 to 28°C leads to a raise in production of MCFAs, while at 32°C the MCFA level decreased being the lowest among the variants. Also, the biomass resulted at 28°C was the highest while at 32°C was the lowest, which confirms that the temperature is an important factor able to affect yeast growth and metabolism, consequently, altering the final concentration of MCFAs in wines (Restrepo et al., 2019).
However, for white wine fermentation, the results of other authors who experimented with *Saccharomyces cerevisiae* and *Saccharomyces bayanus* revealed opposite results for both yeasts, such as, the production of higher concentrations of MCFAs and MCFAEEs at 13°C as compared to those obtained at 25°C wine samples where the concentrations were lower. In the same study, the fatty acid composition in yeast cells at the end of AF, showed that the yeast membranes contained more MCFAs when they were grown at higher temperatures and more unsaturated fatty acids at lower temperatures, as an adaptive response (Torija et al., 2003a).
Fermentation of several grape juice samples with two yeast strains of *Saccharomyces cerevisiae* (IAM4274 and IAM4268) under controlled temperatures ranging from 10 to 30°C with incremental rises of 5°C showed a decreasing production of MCFAs (expressed as sum of C_6+C_8+C_{10}) in direct correlation with the fermentation temperature increase, while, the C_6 and C_8 showed a decreasing level at elevated temperatures, while C_{10} remained almost constant (Takashi, 1986).
These inconsistencies among results could be attributed to the different levels of oxygen exposure, nutrients or inoculum sizes during experimentation. In one study, using *Saccharomyces cerevisiae* fermenting concentrated grape juice diluted to 200 g/l sugars, it was revealed that the maximum population of yeasts (biomass) resulted at 30°C and the lowest at 35°C, suggesting a higher rate of cell death over 30°C (Torija et al., 2003b). Although, while during AF at temperatures under or equal to 20°C the yeast growth rate after 7 days of fermentation decreased only slightly, at temperatures over 20°C the decrease was more accentuated and at 35°C the decrease was very rapid, indicating a high rate of cell death (Torija et al., 2003b). However, the inhibitory effect of MCFAs was found to be higher at low and intermediate temperatures in the presence of high concentrations of ethanol during AF (Viegas & Sá-Correia, 1997).

The effect of yeast assimilable nitrogen

The consumption of nitrogen is different among different yeast strains and is mostly influenced by the temperature of fermenting media. At higher temperatures the yeasts require more YAN and other nutrients in order to support the growth and produce biomass and as well as volatile compounds (Vilanova et al., 2007). In one study it was shown that *Saccharomyces cerevisiae* grown at 13°C consumed 59 mg/l NH_4^+ and 59 mg/l amino acids, while at 25°C consumed 79 mg/l NH_4^+ and 67 mg/l amino acids (Beltran et al., 2007). The addition of inorganic sources of nitrogen as ammonium cation is strongly influencing MCFAs and MCFAEEs concentrations in the final wines (Vilanova et al., 2007). The timing of ammonium sulphate addition and the dosage influenced mostly the MCFAs production, the additions at the beginning of fermentation (before inoculation) increasing the most the production of MCFAs, while the additions at the halfway point (when 50% of the sugar had been consumed) decreased or only slightly increased the concentration of MCFAs, depending on yeast strain of *Saccharomyces cerevisiae* (Hernandez-Orte et al., 2006). The additions of ammonium sulphate at the beginning of fermentation influenced less the MCFAEE (ethyl hexanoate and ethyl octanoate) production, the doses of 260 mg/l NH_4^+ tending to only slightly increase the final concentration of MCFAEEs, while doses of 400 mg/l NH_4^+ left the production of fatty acid ethyl esters at about the same level as in the control samples with only 120 mg/l NH_4^+, depending on yeast strain (Hernandez-Orte et al., 2006). The concentrations of MCFAs and MCFAEEs were observed to be increased in Chardonnay wines obtained from musts purposely selected for their low nutrient and sugar content (180 g/l). When fermented with *Saccharomyces cerevisiae* yeast strain Vinoferm Crio, the addition of inorganic or complex nutrients in musts during AF decreased significantly their concentration in a dose-dependent manner (Csutoras et al., 2022).

It was also observed that a high YAN concentration in the fermenting media can decrease the production of hexanoic acid and ethyl hexanoate, suggesting a strong influence of the used yeast strains on the production of this compound (Hernandez-Orte et al., 2006; Hernández-Orte et al., 2005).

Supplementation of musts with several amino acids (*β*-alanine, cysteine, arginine and valine) with doses which fall within the normal concentrations found in grapes, or slightly higher than those, was investigated to determine their potential role in CoA biosynthesis pathway in the *Saccharomyces cerevisiae*, after normalizing YAN concentration by addition of ammonium chloride (NH4Cl) (Boss et al., 2015). *β*-alanine, a pantothenate (B5 vitamin) precursor in the pathway of CoA biosynthesis, was the only amino acid shown to increase the production of MCFAs (hexanoic, octanoic and decanoic acids), MCFAEEs (hexanoate, octanoate and decanoate ethyl esters) and acetate esters (ethyl acetate, 2-phenylethyl acetate and isoamyl acetate) in the final wines (Boss et al., 2015). The stimulatory doses for the production of MCFAs and MCFAEEs were found to be around 1 mg/l, but the addition of more *β*-alanine, even up to 100 mg/l, does not significantly increase this production further (Boss et al., 2015).

The effect of pH

Organic acids of wines are generally derived from grapes, corrections made during processing or from microbiological activities occurring before, during or after AF, including in MLF (Chidi et al., 2018). Usually, organic acids determine basic wine parameters, such as titratable acidity and pH, which vary with the grape variety, harvesting time, vintage or terroirs. The pH of grape musts and wines range between 3.0 and 4.0 and frequently requires adjustments, especially in hot climates, due to its importance for wine microbial stability, flavour and aroma (Chidi et al., 2018). Grape musts which underwent pH adjustments in the range of 2.8 to 4.5 revealed an increased in production of MCFAs (expressed as sum of C_6+C_8) at higher pH values and a decline at lower values, while the C_8/C_6 acids ratio showed a decreasing trend with an increase in pH value (Takashi, 1986). Decanoic acid was not detected in pH-adjusted grape musts and therefore no trends are known for this acid (Takashi, 1986). Usually, the MCFAEEs production is correlated with the concentration of MCFAs precursors (Takashi, 1986). These esters are found to be relatively unstable in wines, especially in low pH wines which go through a storage period at elevated temperatures, which is favouring their hydrolysis (Ramey & Ough, 1980). Fermented grape musts with higher pH values tend to contain and preserve more MCFAEEs, due to an increased production of MCFAs during AF (Takashi, 1986) and of CoA, the hydrolysis being especially slowed down when the wine storage is short and done at low controlled temperatures (Ramey & Ough, 1980).

The effect of different yeast strains

The effect of yeast strain on MCFAs and MCFAEEs production is of much importance. A comparison of two yeast strains of *Saccharomyces cerevisiae* popular for white wine production in Australia, AWRI 796 and M05, revealed that the strain AWRI 796 produces 3 to 4 times more MCFAs than the strain M05 in the same conditions of nitrogen adjustments with inorganic nitrogen, when YAN levels ranged from 117 to 500 mg N/l (Vilanova et al., 2007). The production of hexanoic acid is strongly influenced by the *Saccharomyces cerevisiae* yeast strain and YAN levels. Hexanoic acid decreased in samples with high YAN and fermented with

AWRI 796 strain, while this trend was not observed for the strain M05 (Vilanova et al., 2007). In a similar study, some strains (Fermicru AR2 and Stellevin NT116) produced less ethyl hexanoate at high YAN levels, while others (LW LVCB) produced more of this compound (Hernandez-Orte et al., 2006; Hernández-Orte et al., 2005). It was suggested that the MCFAEEs synthesis is dependent on MCFAs substrate concentrations and that an increase in ethyl esters is expected when the MCFAs are added during the AF (Saerens et al., 2006). However, the addition of C_6 or C_8 fatty acids during AF using a wild type strain *BY4741* and constructed strains with overexpression of *BY4741+ pEHT1s*, *BY4741+ pEEB1s* and *BY4741+ pYMR210ws* increased the concentrations of the respective MCFAEEs but, no significant differences were found between the wild type strain and overexpression strains (Saerens et al., 2006).

Several studies conducted on different yeast strains and different levels of adjusted YAN, revealed that production rates of MCFAs and MCFAEEs are also strongly dependent on yeast strains, not only on YAN level (Hernandez-Orte et al., 2006; Hernández-Orte et al., 2005; Vilanova et al., 2007). Other yeasts, such as *Lachancea thermotolerans*, known to be low producers of MCFA and MCFAEEs, are used to avoid strong smells given by fatty acids during red wine production, improving the red wine aroma (Hranilovic et al., 2021; Shekhawat et al., 2017). In Table 1 are presented the ranks of odor activity values (rank 1 being the lowest and rank 6 the highest) for MCFAs and MCFAEEs in wines produced by mixed fermentations, using several non-*Saccharomyces* yeasts and *S. cerevisiae.* Compared to *S. cerevisiae*, the production of MCFAs increases in the case of non-*Saccharomyces* yeasts such as *Metschnikowia pulcherrima*, *Candida stella*, and *Pichia fermentans* (Liu et al., 2016). Opposite results were reported for *Hanseniaspora uvarum* and *Issatchenkia orientalis* in mixed fermentations (Hu et al., 2018; Liu et al., 2016).

Table 1. Ranks* of odor activity values (OAV) for MCFAs and MCFAEEs in wines produced by mixed fermentations with non-*Saccharomyces* plus *S. cerevisiae* compared with monoculture of *S. cerevisiae* (Liu et al., 2016)

Compounds	*Saccharomyces cerevisiae*	*Hanseniaspora uvarum* + *Saccharomyces cerevisiae*	*Metschnikowia pulcherrima* + *Saccharomyces cerevisiae*	*Candida stella* + *Saccharomyces cerevisiae*	*Pichia fermentans* + *Saccharomyces cerevisiae*	*Issatchenkia orientalis* + *Saccharomyces cerevisiae*
C6-MCFA	6	1	2	4	3	5
C8-MCFA	1	3	4	6	5	1
C10-MCFA	2	3	4	5	6	1
Total MCFA	**3**	**1**	**4**	**6**	**5**	**2**
C6-MCFAEE	4	1	3	5	6	2
C8-MCFAEE	3	1	4	6	5	2
C10-MCFAEE	5	1	4	2	6	3
C12-MCFAEE	4	1	2	6	5	3
Total MCFAEE	**4**	**1**	**3**	**5**	**6**	**2**

*Ranks are ascending, with 1 being the lowest and 6 the highest

The different metabolic pathways activated in yeasts and their effect on the production of MCFAs and MCFAEEs are also important for the red wine production, as these compounds can inhibit the growth of *O. oeni* and impair MLF (Balmaseda et al., 2018). The C_8-C_{14} MCFA can inhibit the growth of *O. oeni* and reduce the consumption of L-malic during MLF (Edwards & Beelman, 1987; Lonvaud-Funel et al., 1988). In mixed fermentations, time of yeast inoculation showed a strong influence on MCFA and MCFAEE production, as revealed in experiments with *H. uvarum* and *S. cerevisiae* (Hu et al., 2018). The increased MCFA concentration is, as expected, directly correlated to an increased MCFAEE production (Hu et al., 2018). Inoculation with *H. uvarum* with 48 h before *S. cerevisiae,* led to increased concentrations of MCFAs and MCFAEEs in wines, while simultaneously inoculation or earlier inoculation (96 h) of *H. uvarum* showed decreased concentrations of these compounds (Hu et al., 2018). The production of C_6-C_{10} saturated fatty acids were significantly different among various yeasts species and strains tested on sterile filtered grape must, fermented at 20°C in hermetic vessels equipped with fermentation stoppers (Takashi, 1986). The yeast species and strains generally produce different total MCFA concentrations expressed as sum of C_6+C_8+C_{10} of fatty acids, even when they ferment the same media, in the same conditions. Since C_{10} content appears to be affected to a lesser extent, for a better characterization of the MCFA production by yeasts the C_6/C_8 ratio can be used (Takashi, 1986).

PROPERTIES OF MCFAs AND THEIR ETHYL ESTERS

Medium chain fatty acids (MCFAs) and their esters (MCFAEEs) are important volatile compounds, which contribute to sensorial profile of wine. The aroma descriptors of several MCFAs and MCFAEEs are presented in Table 2, along with other properties and their concentration range in wine. The MCFA aroma is generally of fatty type, while MCFAEE aroma is of fruity type (Liu et al., 2016), therefore higher concentrations of MCFAEEs and lower concentrations of MCFAs are desired in wines, but this is only partially obtainable, as the ethyl esters concentration is dependent on the concentration of MCFA, which act as precursors. The MCFAs and MCFAEEs are lipid soluble compounds, which can diffuse through the yeast membrane into the fermenting wine (Nykänen & Nykänen, 1977; Saerens et al., 2010). As compared to acetate esters, for which the excretion occurs rapidly and completely, the transfer of ethyl esters to fermentation media is slower and depends on the length of carbon chain, the bigger the chain the slower the transfer. Generally, the excretion of esters is reported to be 100% for C_6, 54-68% for C_8 and 8-17% for C_{10} (Nykänen & Nykänen, 1977; Saerens et al., 2010).

Table 2. Properties and concentrations of MCFA's and their ethyl esters in wines

Identification of compounds	Chemical properties	Sensorial descriptors	Odor threshold (*model wine), mg/l	Wine concentration range (average), mg/l
Medium-chain fatty acids (MCFAs)				
Hexanoic acid (C_6) (syn. caproic acid) CAS 142-62-1	**Solubility in water (25°C) g/l**[5] = 10.285 **pKa (25°C)**[1] = 4.880 **O:C ratio** = 0.44	goat-like, cheese, sour, fatty, sweat	[29]0.29; [8, 30]0.42; [9, 10, 25]3.00; [26]5.40	White wines: n.d.-25.08 (**3.640**)[12, 13, 14, 15, 16, 17] Red wines: 0.217-3.782 (**1.262**) [8, 21, 22, 23, 24]
Octanoic acid (C_8) (syn. caprylic acid) CAS 124-07-2	**Solubility in water (25°C) g/l**[6,7] = 0.73483 (Calculated from the solubilities at 20 and 30°C) **pKa (25°C)**[2]=4.895 **O:C ratio** = 0.33	cheese, sweat, soapy, waxy	[8, 30]0.50; [10, 25, 29]3.00; [26]5.80	White wines: n.d.-34.7 (**7.230**) [12, 13, 14, 15, 16, 17] Red wines: 0.194-14.536 (**2.181**) [8, 21, 22, 23, 24]
Decanoic acid (C_{10}) (syn. capric acid) CAS 334-48-5	**Solubility in water (25°C) g/l**[7] = 0.06184 **pKa (25°C)**[3] =4.900 **O:C ratio** = 0.27	citrus, sour, fatty, unpleasant, rancid, soapy	[8, 30]1.00; [26]3.50; [10, 25, 29]10.00; [9]15.00	White wines: n.d.-29.62 (**2.570**) [12, 13, 14, 15, 16, 17] Red wines: 0.039-0.857 (**0.290**) [8, 21, 22, 23, 24]
Dodecanoic acid (C_{12}) (syn. lauric acid) CAS 143-07-7	**Solubility in water (25°C) g/l**[7] =0.00324 **pKa (20°C)**[4]=5.300 **O:C ratio** = 0.22	dry, metallic, laurel oil flavour	[11]1.00; [26]10.00	White wines: n.d.-1.24 (**0.240**) [12, 13, 14, 15, 16, 17] Red wines: not found
Medium-chain fatty acid ethyl esters (MCFAEEs)				
Ethyl hexanoate (syn. ethyl caproate) CAS 123-66-0	**Solubility in water (25°C) g/l**[19, 20]= 0.6290 **O:C ratio** = 0.33	green apple, banana, wine, pineapple	[27, 28] 0.001; [9, 30]0.005; [8, 30]0.014; [30]0.023; [10]0.062	White wines: 0.0011-1.636 (**0.502**) [12, 13, 14, 15, 16, 17, 24] Red wines: 0.052-0.974 (**0.342**) [8, 21, 22, 23, 24]
Ethyl octanoate	**Solubility in water (25°C)**	pear,	[9, 30]0.002; [8,]	White wines:

Identification of compounds	Chemical properties	Sensorial descriptors	Odor threshold (*model wine), mg/l	Wine concentration range (average), mg/l
(syn. ethyl caprylate) CAS 106-32-1	**g/l**[19, 20]= 0.0701 **O:C ratio** = 0.27	pineapple, floral, apricot	[30]0.005; [30]0.019; [29]0.070	0.0012-2.770 (**1.392**) [12, 13, 14, 15, 16, 17, 24, 33] Red wines: 0.034-0.783 (**0.318**) [8, 21, 22, 23, 24]
Ethyl decanoate (syn. ethyl caprate) CAS 110-38-3	**Solubility in water (25°C) g/l**[19, 20]= 0.0159 **O:C ratio** = 0.22	grape, pear, oily, sweet, waxy, fruity, apple, soapy, winey	[30]0.005; [29]0.122; [8, 30]0.200	White wines: 0.0014-2.800 (**0.557**) [12, 13, 14, 15, 16, 17, 24, 33] Red wines: 0.015-0.470 (**0.078**) [8, 21, 22, 23, 24]
Ethyl dodecanoate (syn. ethyl laurate) CAS 106-33-2	**Solubility in water (25°C) g/l**[19, 20]= 0.0004 **O:C ratio** = 0.19	pear, fruity, floral, leaf	[11]3.500; [32]5.900	White wines: 0.0002-0.202 (**0.042**) [14, 15, 16, 24, 33] Red wines: 0.006-0.037 (**0.018**) [8, 20, 21, 22, 23]

[1](Riddick et al., 1985); [2](Dean, 1987); [3](Barratt, 1996); [4](Serjeant & Dempsey, 1979); [5](Yalkowsky, 2010); [6](O'Neil, 2006); [7](Yalkowsky & Dannenfleser, 1992); [8](Ferreira et al., 2000) - *determined in 11% v/v aqueous ethanol with 7 g/L glycerol, 5 g/l tartaric acid and pH adjusted to 3.4 with 1M NaOH; [9](Guth, 1997) - *determined in 10% w/w aqueous ethanol; [10](Gemert, 1999) - *odour threshold values in water; [11](Sun & Liu, 2004); [12](Csutoras et al., 2022); [13](Li et al., 2008);[14](Kim et al., 2018); [15](Estévez et al., 2004); [16](Vázquez-Pateiro et al., 2020); [17](Vázquez-Pateiro et al., 2022); [18](HMDB, 2023); [19](FooDB, 2023); [20](Slaghenaufi et al., 2021); [21](Geffroy et al., 2020); [22](Zhao et al., 2017); [23](Noguerol-Pato et al., 2014); [24](Philipp et al., 2018); [25](Buttery et al., 1988); [26](Fazzalari, 1978); [27](Takeoka, 1990); [28](Siek et al., 1969); [29](Abilleira et al., 2010); [30](Giri et al., 2010); [31](Francis & Newton, 2005); [32](Lyu et al., 2019); [33](Rodríguez-Bencomo et al., 2002).

TOXICITY OF MCFAs ON WINE MICROORGANISMS

The effect of MCFA chain length and concentration

As already mentioned, the C_6, C_8, C_{10} and C_{12} saturated fatty acids are metabolites produced by yeasts during AF which act as inhibitors for yeasts and bacteria. The MCFA mixture (C_6, C_8 and C_{10}) in the fermentation media exerts a stronger inhibition than the one produced by single acids in the same concentration, meaning that the MCFAs in combinations have synergic inhibitory effect (Lonvaud-Funel et al., 1988).

The length of carbon chain was found to be crucial for yeast toxicity, the longer the chain, the more liposoluble the acid (Table 2). Liposolubility facilitates the entrance of the undissociated acid through membranes, the molecules more liposoluble becoming toxic at lower concentrations (Borrull et al., 2015). The passive diffusion through the cytoplasmic membrane of the MCFAs produced by the yeasts is inversely proportional to fatty acid chain length and thus the equilibrium between the MCFAs inside of a cell and the outside fermentation media is governed by this process (Bardi et al., 1999). The C_6 and C_8 concentrations are practically constant inside cells, while, the concentrations in fermentation media rise to very high concentrations during growing phase. For C_{10} was found to be relatively abundant both in the cell and in the fermentation media, and for C_{12} was found that it is present almost exclusively in the cell, due to the slow diffusion process (Bardi et al., 1999). This process revealed that MCFAs do not replace UFAs in the membrane to maintain its fluidity status (Bardi et al., 1999), but actually tend to remain blocked across the membrane when cell tries to excrete them. The result is an increase in membrane fluidity and an acidification of the cytosol by the organic acids which remain in the cell. This process explains why the inhibitory properties increase with the increase of chain length.

It was established that undissociated MCFAs with longer chains, C_{12} and C_{14}, are the most toxic for *Oenococcus oeni* and therefore may represent a serious problem for red winemaking, where starting MLF is necessary (Guilloux-Benatier et al., 1998). However, the esterified forms (MCFAEEs) of C_{10}, C_{12}, and C_{14} are found to be even more toxic than the free fatty acids for *Oenococcus oeni* (Guilloux-Benatier et al., 1998). The concentrations of octanoic acid up to 16 mg/l and decanoic acid up to 8 mg/l increased the duration of growth latency and decreased exponentially the maximum specific growth rate and the biomass yield in the presence of 12 and 14% v/v ethanol (Viegas & Sá-Correia, 1997). An increased toxic effect of these tested concentrations was observed with the pH decrease and with the temperature increase (Viegas & Sá-Correia, 1997). The concentrations of decanoic acid between 5 and 10 mg/l inhibited the growth of *Oenococcus oeni,* while doses of 30 mg/l induced rapid bacterial death (Edwards & Beelman, 1987). In another study (Lonvaud-Funel et al., 1988), 4 mg/l decanoic acid or 0.5 mg/l dodecanoic acid were able to inhibit MLF when independently tested. Therefore, it is clear that both the concentration and the carbon chain length of the used MCFA determines the extent of the inhibitory/lethal effect on yeasts and bacteria (Borrull et al., 2015; Edwards & Beelman, 1987; Viegas & Sá-Correia, 1997). Interestingly, for lactic bacteria, decanoic acid up to 12.5 mg/l and dodecanoic acid up to 2.5 mg/l were found to act as growth factors in the presence of low ethanol concentration (4% v/v) in synthetic media with pH=4.5 (higher pH in wine), stimulating their growth activity; however, at higher concentrations the same acids exerted an inhibitory effect (Capucho & San Romão, 1994). Due to the interaction between various stress-inducing factors, such as ethanol concentration and pH of the growth media, the evaluation and prediction of MCFA inhibitory effect is more difficult than expected. Yeasts can tolerate higher concentrations of MCFA. For *Saccharomyces cerevisiae* BY4742 grown in a medium with pH=5.8 and ethanol under 0.5% v/v the non-inhibitory concentrations (NIC) were 35 mg/l for C_8 and 25 mg/l for C_{10}, while the minimum inhibitory concentrations (MIC) were 125 mg/l for C_8 and 80 mg/l for C_{10}, respectively. As expected, C_{10} is more toxic than C_8. Compared to a control sample, the viability of *S. cerevisiae* BY4742 in the presence of MCFA was reduced to 54.9% for C_8 and 42.3% for C_{10} at NIC and to 14.7% for C_8 and 6.5% for C_{10} at MIC. However, for this strain cultivated in YPD media incubated overnight semi-anaerobically at 28°C and pH 5.8, as compared to the acids used independently, the combination of C_8+C_{10} fatty acids led to an increased vitality at NIC and a decreased one at MIC levels, showing that for the combination of C_8+C_{10} the effect is not additive, but synergic (Borrull et al., 2015). Similarly, the inhibitory effect of MCFAs on yeast growth studied through a calorimetric technique was also determined to be correlated with the chain length of MCFA. At pH=5.5 and ethanol concentration of 0.66% v/v the 50% inhibitory concentrations for two anaerobic yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) and two aerobic yeasts (*Candida utilis* and *Kluyveromyces marxianus*) ranged between 62 to 81 mg/l C_8 acid and from 30 to 86 mg/l for C_{10} acid. The determined MIC values ranged between 159 to 239 mg/l for C_8 acid and between 77 to 127 mg/l C_{10} acid (Antoce et al., 1998; Antoce et al., 1997).

The effect of ethanol on MCFA inhibitory properties

Ethanol, an important component in wine, has its own antimicrobial properties, but together with other compounds the effect can synergistically increase. A 10 to 80 mg/l of an MCFA mixture (C_8:C_{10}:C_{12} in 2:7:1 ratio dissolved in 70% v/v ethanol) was tested at pH=3.5 on several yeast species (*S. cerevisiae*, *S. uvarum*, *Starmerella bacillaris* and *Zygosaccharomyces bailii*). The study showed that at low ethanol concentrations of about 5% v./v., without added SO_2, the added MCFA mixture, even at the highest dose applied, was not enough to completely inhibit the growth of *S. cerevisiae* and *Zygosaccharomyces bailii* (Horváth et al., 2020). In the same study, an attempt was made to prevent the refermentation of Tokaj Essence (2.32% v/v alcohol; 0/38 mg/l free/total SO_2 and 54% w./w. total soluble solids) by adding 40 mg/l MCFA mixture and 100 mg/l SO_2 and incubating it 15°C. The test also revealed that in the absence of higher amounts of ethanol, the viable yeast cell numbers remained of about 10 CFU/ml, which is considered a non-acceptable risk of refermentation for a bottled wine. The study concluded that the application of MCFAs to control the growth of microorganisms should only be used when significant amounts of ethanol are also present (Horváth et al., 2020). In the presence of increasing levels of ethanol, the inhibitory effect of decanoic acid (Antoce et al., 1998) or of other organic acids is synergistically increased (Antoce et al., 1997). Thus, it was proven that high ethanol concentrations enhance the inhibitory properties of MCFAs (Antoce et al., 1998;

Antoce et al., 1997), while in low ethanol products a reduced toxicity of MCFAs is observed (Horváth et al., 2020).

In the presence of MCFAs, the tolerance of yeast to ethanol is also reduced. A good ethanol tolerance is dependent on the composition of cell membrane, which should contain more lipids with higher degree of unsaturation (UFAs). For this reason, yeasts adapt continuously to the content of ethanol produced during fermentation (Arneborg et al., 1995; Costa & Moradas-Ferreira, 2001; Piper, 1995). If these lipids are not present in the fermentation media, for the synthesis of UFAs and ergosterol, oxygen exposure is required at the end of the exponential phase of growth (Chen, 1980; Ding et al., 2010; Lafon-Lafourcade et al., 1979). Another observation which supports this theory of ethanol tolerance being dependent on the yeast membrane composition is that the presence of high ethanol concentrations during the mid or the end of AF leads to an increase in the fluidity of yeast membranes; meanwhile, the ergosterol antagonizes the effect of ethanol by increasing the rigidity of membranes (Ding et al., 2009).

The effect of pH on MCFA inhibitory properties

The pH of wines range between 2.8 and 4.2 and usually, the growth of *Saccharomyces cerevisiae* is not affected in this range, but lower values of the pH may affect their growth and AF.

The pH becomes harmful to microorganisms at the lower values of the scale (2.8-3.2), especially combined with high ethanol concentrations (Pampulha & Loureiro-Dias, 1989; 1990). The undissociated forms of organic acids are present in higher percentages at low pHs and the influence of ethanol on membrane fluidity increases the toxicity, allowing passive diffusion across the weakened membrane. One of the mechanisms that explains the cell death relys on the cytosol acidification through passing of undissociated MCFA across the cell membranes into the neutral cytoplasm by means of passive diffusion (Viegas & Sá-Correia, 1997). Upon entering the cells, these acids start to dissociate in cytosol, as their pKa is relatively low compared to the cytosol pH, contributing to a decrease of the internal pH (Capucho & San Romão, 1994; Pampulha & Loureiro-Dias, 1989; 1990). As a result, the acidification of the cytosol can lead to sluggish or stuck fermentation. In fermentation media the toxicity of MCFAs was proven to be increased when the pH is decreasing (Antoce et al., 1998). Potassium ions in the fermentation media can counteract the harmful effect of acidic pHs, increasing the tolerance of yeasts (Kudo et al., 1998).

The MCFAs are found undissociated in the musts or wines with low pH, as shown in the Figure 1. Even at higher pH values, of up to 4.2, their undissociated forms represent 80-95%. This mechanism of passive diffusion of the undissociated forms of acidic compounds is often exploited in oenology to produce antimicrobial effects, being valid for various acidic preservatives, including sulphites.

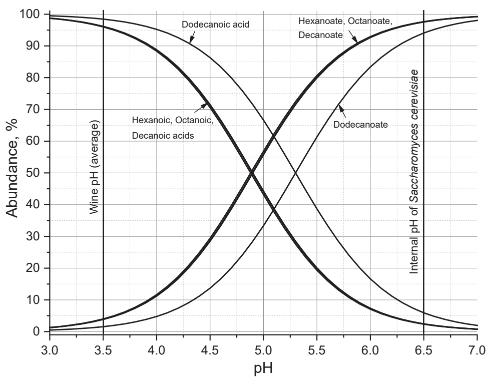

Figure 1. Dissociation of MCFA depending on pH at 25°C

The effect of temperature on MCFA inhibitory properties

Temperature of growth media enhances the inhibitory effect of MCFAs especially during the mid or the end of AF, due to increased ethanol accumulation and increased fluidity of cell membranes (Ding et al., 2009). The lack of oxygen at the end of exponential growth phase of yeasts or absence of ergosterol and UFAs in fermentation media weaken yeast membranes, which are prone to stress factors (Costa & Moradas-Ferreira, 2001; Lafon-Lafourcade et al., 1979; Piper, 1995). During white or rose wine production, where lower temperatures are implemented during AF, the viability for *Saccharomyces cerevisiae* is expected to be not as affected as in the case of higher temperatures (Viegas & Sá-Correia, 1997), but actually, the overall loss of viability is higher, due to other conditions created in these winemaking processes, such over-clarified musts, anaerobic fermenting media, etc. Some interventions are possible to use to counteract the loss of viability, as mentioned before, stimulating the incorporation in the yeast membrane of ergosterol and UFAs (Chen, 1980) from the nutrients added at rehydration. During red wine production, where AF of must takes place on grape skins, higher temperatures are required to facilitated the extraction of pigments and tannins, and, as a consequence, the viability of *Saccharomyces cerevisiae* is expected to decrease (Viegas & Sá-Correia, 1997), but actually the loss of viability is here counteracted by the other conditions used in red winemaking, such as the presence of UFAs from the contact with the grape fragments, the presence of higher levels of oxygen introduced during pumping-over etc. Thus, the effect of temperature and modulation of yeast cell viability should be considered in connection with the other factors during winemaking.

Yeast adaptation and resistance to MCFA toxicity

Generally, yeasts adaptation and improvement in resistance to inhibitors mainly involves cell detoxification through transmembrane transporters, aiming to excrete the toxic compounds out of the cell, but other mechanisms are not excluded (Balzi & Goffeau, 1995; Goffeau et al., 1997). The cells detoxification is rather a complex process, including many, even overlapping, pathways.

The detoxification mechanisms for MCFA, could include plasma membrane H^+-ATPase induction, MCFAEEs production, activation of a membrane transporters for the MCFA corresponding anions or activation of beta-oxidation pathway in peroxisomes (Borrull et al., 2015; Cabral et al., 2001; Legras et al., 2010).

Weak acids could induce plasma membrane H^+-ATPase transporters (proton pump). Through this, under mild stress, *Saccharomyces cerevisiae* regulates the cytosol pH. The exposure of yeast cells to C_8 fatty acid or to ethanol was found to induce this transporter to translocate protons out of the cytosol, but an octanoate active transporter from the cytosol to the surrounding medium has not been identified (Cabral et al., 2001). However, in case of a rapid exposure to C_8 fatty acid, the unadapted cells of *Saccharomyces cerevisiae* are able to activate *de novo* protein synthesis and produce a plasma membrane transporter, which mediates the active efflux of octanoate out of the cell (Cabral et al., 2001).

For octanoic acid another transporter was also found in yeasts. The induction of Pdr12p transporter confers yeast resistance to several weak acids, including sorbic and benzoic acids, allowing the expulsion of the anions from the cytosol (Holyoak et al., 1999; Piper et al., 1998). This transporter was initially found to work on monocarboxylic acids with an aliphatic chain shorter than C_7 (Holyoak et al., 1999), but later was discovered that Pdr12p transporter may be induced for C_8 acid expulsion as well (Legras et al., 2010), because the length of C_8 acid is relatively close to the one of short-chain organic acids and induces a similar response.

Esterification of MCFAs to MCFAEEs could be considered a possible yeast detoxification metabolism, which requires the expression of *EHT1* and *EEB1* genes, but also combined with *ATF2*, which is expressed under anaerobic conditions and is known for its role in the acetylation of sterols (Cauet et al., 1999; Saerens et al., 2010). *EEB1* gene expression responds differently to the supplementation of C_8 or C_{10} fatty acids during AF. An increased expression of *EEB1* gene was observed when C_8 acid supplemented into media, while this was not observed for C_{10} acid (Legras et al., 2010). This could explain why in wines C_8 ethyl ester is found in higher concentrations as compared with C_{10} ethyl ester (as also shown in Table 1). This metabolic route could contribute more for the C_8 detoxification, than to C_{10} or other longer chain fatty acids.

A major role for resistance to C_{10} fatty acid, which contributes also to C_8 resistance, was attributed to Tpo1p carrier protein, known to protect cells against various inhibitory molecules. Due to this mechanism, it was observed that a high level of C_{10} acid resistance in yeasts is always associated with a high level of C_8 acid resistance (Legras et al., 2010).

Another possible response to improve the yeast resistance against MCFAs could be the membrane adaptation through the induction of Pdr16p transporter (Legras et al., 2010). However, the expulsion of C_8 and C_{10} fatty acids, along with that of many other inhibitors, is mainly determined by the two transporters discussed previously, Tpo1p and Pdr12p. These two transporters represent the main elements of the yeasts resistance to MCFAs (Legras et al., 2010).

Finally, the adaptation of yeasts to MCFAs could involve an oxidative stress response, similar to that observed for other inhibitors, which implies the activation of *β*-oxidation pathway in peroxisomes. The MCFAs are broken down to other non-inhibitory molecules with the formation of hydrogen peroxide, which subsequently is converted by catalase into water and oxygen, to protect the cells from reactive oxygen species (Borrull et al., 2015; Legras et al., 2010).

CONCLUSIONS

The production of MCFAs is triggered by an anaerobic environment and is generally enhanced at higher temperatures during AF. Higher concentrations of MCFAs in fermentation media, in the composition of yeast membranes or in cytosol can become toxic during the middle or the end phase of AF. The toxicity is especially manifest in the case of white and rose winemaking, where an anaerobic environment and low pH are technologically preferred, which coupled with the increasing concentration of ethanol, can lead to slow or stuck fermentative processes. The red winemaking is generally less problematic as far as the MCFA toxicity is concerned, because it is partially countered by the contact of fermenting must with the grape solids containing UFAs and by the higher dissolved oxygen introduced during cap management, especially by means of pump over. To support this, the studies showed that the fermentation on skins of Cabernet Sauvignon produced less MCFA then the clarified must of the same grapes, under the same conditions (Guilloux-Benatier et al., 1998; Takashi, 1986). Under certain circumstances, such as those for red fruity wine production, where the maceration is shorter and oxygen level is lower during the fermentation, MCFA concentrations are generally higher and may inhibit the activity of lactic acid bacteria and, consequently, the onset of MLF. The yeast strains differ very much in their abilities to produce MCFAs and MCFAEEs, their response being variable depending not only on their genetic characteristics, but also on the intrinsic and extrinsic factors affecting the fermentation environment and fermentative performance. The strains proposed for red wine production are generally selected in order to produce as few as possible MCFAs during AF, so that they will not impair later on the development of lactic bacteria and onset of MLF. In white wines, yeasts which produce moderate MCFAs and MCFAEEs can be interesting for the final fruity aroma of wines. In the case of high levels of MCFAs in the must, the esterification of MCFAs during AF can be considered a defence mechanism developed by yeast to protect themselves against the toxicity of medium-chain saturated fatty acids, but other overlapping mechanisms, such as induction of several membrane transporters to excrete the acids or the protons from the cells or *β*-oxidation pathway for MCFA degradation can also be involved for cell adaptation and resistance.

Knowing all these complex mechanisms and depending on what kind of effects the winemaker wants to trigger in relation to the MCFA production or toxicity, various types of yeast metabolism modulation are, within limits, possible.

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STUDY ON THE IMPACT OF CLIMATE CHANGES ON THE PHENOLOGY AND ADAPTABILITY OF SOME VARIETIES INTENDED TO PRODUCTION QUALITY RED WINES

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Abstract

If until two or three decades ago, the limit of cultivation of varieties for red wines was located mainly in the regions of the southern half of our country, and only insularly in a few northern areas, as a result of the increase in thermal resources and solar radiation, this has expanded considerably, at the level of the entire country at the level of the entire country. In the medium term, the choice of varieties for red wines, when establishing new plantations in the north of the country, and not only, can be made from the existing international assortment, already tested, from the local Viticultural germplasm, but in the long term the main solution is to cultivate the varieties new, created to better cope with climate change. The present paper aimed to follow the behavior of Cabernet cubin, Cabernet dorio and Cabernet dorsa varieties, not cultivated on a large scale so far in our country. The results showed that they have high biological resistance to frost and cryptogamic diseases, a good choice in the current climate context.

***Key words**: adaptation, climate change, grapevine, genetic variability.*

INTRODUCTION

According to the forecasts of the Intergovernmental Panel on Climate Change (IPCC, 2014), the average global temperature on the Earth's surface could increase in this century by 1.8-4.0°C. In this context, it is important to apply innovative measures to mitigate and combat the negative effects of climate change.

Grapevine is one of the plants most affected by these changes, being subjected more and more to radiation, thermal and water stress, with negative effects on production and especially on its quality.

The studies carried out so far have highlighted in most of the country's vineyards a significant warming, with influence on the unfolding of the vine phenophases, the main physiological processes, vegetative growth, grape production and quality (Irimia et al., 2015; Bucur & Babeș, 2016; Bucur & Dejeu, 2017). Also, it was noticed an increase tendency of the average of daily average temperatures, of the average of daily maximum and minimum temperatures, especially during midwinter; this may increase the deacclimation risk of the grapevine and its vulnerability to the frost that may occur subsequently (Cichi et al., 2021).

Heat waves negatively affect the ripening of grapes, with very high accumulation of sugars, the pronounced degradation of acidity (under 6 g/L tartaric acid), the increase of pH values, the development of atypical aromatic compounds, and it also affects the phenolic maturity (Bucur et al., 2019). Consequently, the resulting wines are less suitable for aging, they have a modified aromatic profile and a weaker color. The high concentration of sugars in the berries is not due to photosynthesis and their translocation from the leaves and woody parts of the plant, but due to concentration as a result of water loss through evapotranspiration (Keller, 2015).

To mitigate the negative effects, viticulture benefits from a series of innovative measures to delay the ripening of the grapes and obtain balanced wines with a medium alcohol content, namely: short-term measures - soil maintenance (Dhanush & Patil, 2020; Buesa et al., 2021), management of the green parts of the plant (Silvestroni et al., 2019; Bucur, 2021), choosing the time of harvest and winemaking

techniques; medium-term - orientation of the rows, the choice of land for planting, the use of suitable cultivars and rootstocks (Carvalho et al., 2020), and in the long term - the use of irrigation's, even late ones, obtaining new cultivars more adapted to these conditions (Caccavello et al., 2019; Miras-Avalos & Araujo, 2021).
In addition to the negative effects, the variability of the climate has also generated positive effects (the northward expansion of grapevine culture, at higher altitudes).
Comparatively analyzing the evolution and spatial distribution of the oenoclimate aptitude index (IAOe, Teodorescu et al., 1987), in the period 1961-1990, respectively 1991-2013, Irimia et al. (2017), observed that the areas cultivated with varieties for red wine they expanded in the northern part (Moldova), in the west of the country and the Black Sea coast (Figure 1). As a result of this, in recent years, red wine grape varieties have been introduced in the northern regions, while in the southern regions, the composition of the grapes of the same varieties has experienced excessive levels of sugar and lower acidity.
Today, more than ever, global warming is forcing grapevine adaptation strategies in most, if not all, vineyards in the world (Duchêne et al., 2010; Duchêne, 2016; De Cortázar-Atauri et al., 2017), leading to the changing of the maps of wine regions, and these changes will determine a greater attention directed to the establishment of new plantations.
Practically, in wine-growing areas where drought is more severe, the choice of suitable varieties is a priority, being a battle front against the impact of climate change, but it also ensures the reduction of the inputs necessary for the management of the plantation throughout its life cycle.
This choice can be made either by testing already existing varieties both from the existing international assortment, but also from the local viticultural germplasm (Dobrei et al., 2015; Dinu et al., 2021). In recent years, priority has been given to grape varieties for red wines, which gain in quality, the high heliothermal resources recorded and the relative drought being favorable to them. In other words, the genotype influences the phenology of the plants, and different cultivars of grapevine can have a significantly different length of the vegetative cycle depending on the area (Rustioni et al., 2014a; 2014b; Maghradze et al., 2012, Maghradze et al., 2015).

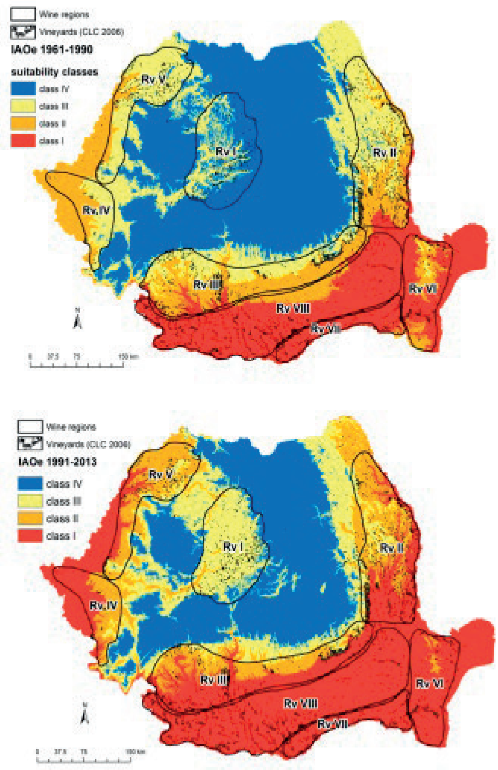

Figure 1. The evolution and spatial distribution of the oenoclimate aptitude index (IAOe), in the period 1961-1990, respectively 1991-2013 (Irimia et al., 2017)

The diversity of vine varieties is an important resource for adapting to climate change (Destrac-Irvine et al., 2020; Antolin et al., 2021).
For this study three table wine red varieties were selected: Cabernet cubin, Cabernet dorio and Cabernet dorsa which are located in the experimental field of the ampelographic collection from the Research and Development Station for Viticulture and Oenology Pietroasa - University of Agronomic Sciences and Veterinary Medicine of Bucharest, an important center for the conservation of the genetic diversity of grapevine and source of Viticultural germplasm, registered in the International Catalog of Ampelographic Collections, under the code indicator "ROM

13", with the right to international exchange of genetic material. These cultivars are not cultivated on a large scale in our country so far and are found only in ampelographic collections, but the current context, of globalization and global warming, determines the monitoring of their agrobiological and technological behavior, and those that prove to be very valuable and that easily adapt to the ecopedoclimatic conditions are to then be cultivated on a larger scale (Stroe, 2020).

MATERIALS AND METHODS

Study area and grapevine cultivars

The research was carried out in the 2019 and 2020 seasons at the University of Agronomic Sciences and Veterinary Medicine Bucharest (N Lat.: 44°47'07"; E Long.: 26°07'28"; alt. 87 m), in the experimental vineyard, on Cabernet cubin, Cabernet dorio and Cabernet dorsa varieties, with the Cabernet Sauvignon - control (C) (Table 1).

The plantation is located on a relatively flat land, and the varieties studied were planted in 2013 at a distance by 2.2 m (inter-row) and 1.2 m (intra-row), with a density of 3787 plant ha^{-1}. The type of pruning applied is double Guyot on the half-stem, and the load distributed on the plant was 30 buds/vine.

During the vegetation period, the shoots were manually directed vertically, and in the last days of July a slight shortening of the shoots was carried out, leaving a canopy height of approximately 1.3 m. The vine was managed without irrigation and a standard disease control program was applied to control downy mildew, powdery mildew and gray rot (*Botrytis*).

Short presentation of Cabernet cubin variety

Is a new variety obtained in 1970 by crossing Blaufränkisch x Cabernet Sauvignon (https://www.wine-searcher.com/ grape-1216-cabernet-cubin), whit late ripening, variety number VIVC - 20004 (Maul & Röckel, 2015). This variety has strong tannic characteristics and rich in flavors berry like Cabernet Sauvignon, and good frost resistance and is suitable for cultivation in regions prone to colder temperatures, but has also been shown to be resistant to oidium, gray rot - like the parent variety Burgund mare (synonymous Blaufränkisch). It is practically the only one of the three analyzed varieties that confirmed its parentage through genetic markers.

Short presentation of Cabernet dorio variety

Is a new red wine variety from Germany, established in 1971 in the cross section of the Blaufränkisch x Dornfelder variety area, Research Institute for Viticulture and Fruit Growing in Weinsberg (Württemberg), (https://www.wine-searcher .com/grape-1217-cabernet-dorio), variety num-ber VIVC - 20003 (Maul & Röckel, 2015). The variety shows great vigor, medium to late ripening and good resistance to low winter temperatures. It produces a velvety red wine with subtly pronounced tannins. It is widely grown in Germany and Switzerland, where the variety raises problems in some years due to its rather late full ripening.

Short presentation of Cabernet dorio variety

Cabernet dorsa is a grape variety for red wines grown in cold climate wine-growing areas (Germany, Belgium, Switzerland), having special performances in Germany and Switzerland. It was obtained in 1971 at the National Institute for Education and Research for Agriculture Weinsberg in Württemberg, by crossing Dornfelder x Cabernet Sauvignon varieties, and since 2003 the variety has received variety protection and has become widespread in culture (https://www.wine-searcher.com/grape-1218-cabernet-dorsa), variety number VIVC - 20002. However, the variety was confirmed with genetic markers as being descended from the crossing of the varieties Blauer Limberger (synonymous Blaufränkisch) x Dornfelder (Maul & Röckel, 2015). It is a medium vigor growth variety with a short growing season of less than 160 days, which ripens its wood well in autumn and shows good resistance to low temperatures over winter. Due to its high sensitivity to powdery mildew, this variety should not be planted in areas where climatic and orographic conditions favor the occurrence of this disease. The wines are rich in tannin, have a cherry flavor and are considered suitable for aging in oak barrels. Due to the dark red color of the wine, it is often used in blends. In 2012 in Germany there were 252 hectares cultivated with Cabernet dorsa, and the trend was increasing.

Table 1. Grape varieties for red wine studied (Bucharest, 2019-2020)

Crt. no.	Cultivar	VIVC* no	Genitors	Color of berry skin
1	Cabernet cubin	20004	Blaufränkisch x Cabernet Sauvignon	blue black
2	Cabernet dorio	20003	Dornfelder x Cabernet Sauvignon**	blue black
3	Cabernet dorsa	20002	Dornfelder x Cabernet Sauvignon**	blue black
4	Cabernet Sauvignon (C)	1929	Cabernet franc x Sauvignon	blue black

*Vitis International Variety Catalogue; **Confirmed by markers: Blaufränkisch x Dornfelder (Maul et al., 2012)

Climatic conditions

For this study, there were used weather data recorded at Bucharest-Băneasa meteorological station for the experimental period (2019-2020), as compared to the reference period (1981 - 2010). A series of climatic data were studied (Table 2), and monthly average temperatures were used to evaluate a set of bioclimatic indices commonly used in viticulture: Huglin index (HI), Winkler index (WI) and cool night index (CNI).

Phenological data

Phenology dates referenced to the stages budburst, flowering, veraison and harvesting maturity, were followed according to BBCH (Bioligische Bundesansalt und Chemische Industrie), modified under the COST Action FA1003 “East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding” (Rustioni et al., 2014). Data were recorded at which 50% of buds, flowers, grapes reached the respective phenological stages (BBCH 008 - budburst; BBCH 605 - flowering; BBCH 801 - veraison and BBCH 809 - berries ripe for harvest). Phenological information was reported for the period 2019-2020 and was expressed as "day of year" (DOY), as the number of days after 1 January.

During the vegetation period, determinations were made on the basis of which the fertility coefficients (absolute, relative) and productivity indices (absolute and relative) were calculated.

Quantitative and qualitative parameters examine in research

The grapes were harvested during the two years of observations between September 6-8. At harvesting, determinations were made on quantitative (bunch weight - g, yield - kg/vine) and qualitative parameters (sugar content - g/L, titratable acidity - g/L tartaric acid, pH, must density - g/cm^3). Sugar concentration in grapes was measured by using an Atago digital refractometer and results were expressed in g/L. Titratable acidity was determined by titrating with 0.1 N NaOH using an Pellet digital biurette, and expresed as g/L tartaric acid.

RESULTS AND DISCUSSIONS

Climatic conditions

The climatic indicators were determined for experimental period (2019-2020) comparatively with reference period (1981-2010) after the recommendations of National Meteorological Administration (Dima et al., 2019). Table 2 shows a warming during the experimentation period, both during the growing season and also in the summer season. Thus, the largest differences are found in the case of maximum annual temperatures (2.3°C), those during the summer (June-August) (1.4°C) and those average annual temperatures (1.3°C). Other climatic parameters which recorded higher values, compared to the reference period (1981-2010), are average temperature in the growing season (IV-X), which increased by 0.42°C and average annual minimum temperature, which registered an increase of 1.4°C.

Both annual total precipitation and the one in the growing season had a small variation. Practically there was a decrease in annual rainfall, with 40 mm, but there was a slight increase in precipitation in the summer season.

Bioclimatic indices (Huglin index, Winkler index and Cool night index) also recorded higher values during the current period, as compared to the reference period, as follows: an increase of 129 units for the Huglin index; an increase of 90 units for the Winkler index; an increase of 0.92°C for the Cool night index.

By its increase, the Huglin index passes from the temperate - warm class HI+1 between 1981-2010, to the warm class HI+2 during the recent period. This also changes the climate profile to which local varieties were adapted and creates the climate context for the growing of new wine grape varieties. The Winkler index current average maintains in the climate profile specific to Regions III, suitable for high production of standard to good quality table wines. A similar evolution for the CI which maintains in the class of very cool nights, less suitable to grapes ripening, but with an increased value that reveal the evolution towards the superior cool night class.

Table 2. The main climatic parameters and bioclimatic indices during the experimentation period (2019-2020) compared to the reference period (1981-2010)

Climatic parameters and bioclimatic indices	Average	Years		Average
	1981-2010	2019	2020	2019-2020
Average annual temperature, ºC	11.55	12.92	12.77	12.85
Average temperature in the growing season (IV-X), ºC	18.07	18.53	18.44	18.49
Average temperature in summer (VI-VIII), ºC	22.50	23.05	22.54	22.80
Average annual minimum temperature, ºC	5.03	6.57	6.27	6.42
Average annual maximum temperature, ºC	17.05	19.28	19.35	19.32
Average maximum temperature in the warmest month, ºC	29.87	29.64	31.06	30.35
Average maximum temperature in summer (VI-VIII), ºC	29.01	30.5	30.38	30.44
Annual total precipitation, mm	608	529	608	569
Total precipitation in the growing season (IV-X), mm	428	385	482	433
Total precipitation in summer (VI-VIII), mm	198	142	292	217
Huglin index (HI)	2346	2458	2492	2475
Winkler index (WI)	1726	1825	1806	1816
Cool night index (CNI)	10.45	10.60	12.13	11.37

The development of the main phenophases

The first effect of temperature rising is on advance of phenological stages of grapevine. The differences in the timing of phenological stages and the interval between them are given to genetic factors, climate and soil conditions, and viticultural practices.

Table 3 shows the average day of the year (DOY) and standard deviations of the four phenological stages of grapevine (budbreak, flowering, veraison and harvest) for the period between 2019 and 2020.

The time of **budbreak** occured on average 109 DOY (April 18), earlier for Cabernet dorio (106) and later for Cabernet cubin (110) and Cabernet dorsa varieties (111). Considering year-to-year variability in budburst, Cabernet dorio exhibited the lowest variability (SD ± 2.14 days) while Cabernet dorsa has the greatest variation (SD ± 5.66 days).

All three varieties have budburst earlier than the control Cabernet Sauvignon (115 DOY, April 24).

Flowering occurred on average at DOY 150 (May 29), earlier for Cabernet cubin (149) and later for Cabernet dorio (152), the differences between the varieties studied and control Cabernet Sauvignon being relatively small. Cabernet dorio exhibited the least year-to-year variation (SD ± 6.36 days) while Cabernet cubin had a highest year-to-year variation (SD ± 12.02 days).

Veraison was registered on average at DOY 214 (August 01) earlier for Cabernet dorio variety (213) and later for Cabernet cubin and Cabernet dorsa varieties (215). Cabernet cubin and dorio had the lowest year-to-year variation of ± 3.54 days while Cabernet dorsa varied by ±4.95 days during 2019-2020.

The average day of the year for **grapes ripening**, for the 2 studied years, was DOY 246 (September 02), with very small differences for between the three varieties (01-03 of September). Cabernet dorio and dorsa exhibited the lowest year-to-year variability (SD ± 4.95 days) and the highest was recorded by the Cabernet cubin variety (SD ± 6.36 days) and Cabernet sauvignon control variety (SD ± 9.19 days). The results concerning the main pheno-logical timing across the growing season shown that there are not always strong relationships between growth events.

Fertility and productivity determinations

Fertility is the ability of varieties to form fruiting organs and is influenced by the variety, the environmental conditions (climate and soil) in which the variety is grown, and the applied technology. According to the values taken by the fertility coefficients (absolute and relative), the varieties studied were distinguished by a medium fertility, falling into the 1.1-2.0 variation class. The highest value was achieved by the Cabernet cubin variety (Fca = 1.94, Fcr = 1.44), followed by Cabernet dorio and Cabernet dorsa (Table 4). All the varieties analyzed recorded higher values than the Cabernet Sauvignon variety (control), both regarding the absolute and the relative fertility coefficient. In terms of productivity (the amount of grapes in grams or kilograms produced per shoot), the Cabernet dorio and Cabernet cubin varieties were the most productive, with an absolute productivity index of 364.77 g/shoot and 318.16 g/shoot, respectively; the last place being occupied by Cabernet dorsa, with 217.6 g/shoot.

The values place the first two varieties in the class of those with high productivity, and Cabernet dorsa belongs to the group of varieties with medium productivity (Pia between 100-300 g). The value of productivity indices is influenced by the variability of annual environmental conditions, as well as by some agrotechnical measures.

The determinations regarding the quality of the raw material for winemaking

Table 4 also shows determinations regarding the quality of the must, for the 3 new red grape varieties, respectively sugar content, acidity, pH and must density.

Table 3. Mean day of year (DOY) of the phenological stages (budburst; flowering; veraison and harvest) and the corresponding standard deviations (SD in days), for Cabernet cubin, Cabernet dorio and Cabernet dorsa varieties (2019-2020)

Varieties	Budburst date (DOY)		Flowering date (DOY)		Veraison date (DOY)		Harvest maturity date (DOY)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Cabernet cubin	110 (April 19)	2.83	149 (May 28)	12.02	215 (August 2)	3.54	247 (September 3)	6.36
Cabernet dorio	106 (April 15)	2.14	152 (May 31)	6.36	213 (July 31)	3.54	246 (September 2)	4.95
Cabernet dorsa	111 (April 20)	5.66	150 (May 29)	10.61	215 (August 2)	4.95	246 (September 2)	4.95
Mean varieties	109 (April 18)		150 (May 29)		214 (August 1)		246 (September 2)	
Cabernet Sauvignon (C)	115 (April 25)	3.54	151 (May 30)	8.48	217 (August 4)	5.66	245 (September 1)	9.19

Table 4. Grapes quantitative and qualitative parameters for varieties analyzed (2019-2020)

Varieties	Fertility coefficient (Fc)		Bunch weight (g)	Productivity indices (Pi)		Yield kg/vine	Sugar content (g/L)	Titratable acidity (g/L tartaric acid)	pH	Must density (g/cm^3)
	absolute	relative		Absolute (g/shoot)	Relative (g/shoot)					
Cabernet cubin	1.94	1.44	164	318.16	236.16	6.26	251	5.7	3.40	1.119
Cabernet dorio	1.86	1.30	193	364.77	250.90	7.51	264	5.0	3.43	1.104
Cabernet dorsa	1.70	1.20	128	217.60	153.60	5.44	295	5.6	4.05	1.095
Cabernet Sauvignon (C)	1.83	1.37	89.5	163.78	122.61	2,149	229	4.2	3.74	1.108

Appreciation of the defining elements of the grape quality of the three analyzed varieties shows that their quantitative and qualitative potential can ensure the obtaining of quality red wines, close to those obtained from their parents, as follows: the Cabernet cubin variety from the parent Cabernet Sauvignon (Stroe & Budescu, 2013), and the Cabernet dorsa and Cabernet Dorio varieties from the Dornfelder and Burgund mare varieties (Stroe & Barcanu-Tudor, 2011). It should be mentioned that, the accumulation of these high amounts of sugars (251 g/L, 264 g/L, 295 g/L) in the context of relatively low acidity (5.7 g/L, 5.0 g/L, 5.6 g/L), is recorded in a fairly favorable area (N Lat.: 44°47'07"; E Long.: 26°07'28"; alt. 87 m), and contextually with the objectives of the study – their

cultivation in somewhat cooler areas, once not suitable for the varieties intended for red wines - as a solution to climate change - they will show a good adaptability. A positive side can be noted in the case of high sugars, as they are a good substrate for the entire metabolism of the plant, being in direct correlation with the accumulation of anthocyanins, at least in the case of the Cabernet cubin cultivar, as a descendant of the Cabernet Sauvignon cultivar, which can have in some years a harvest below the quality standard in terms of the color of the wine obtained. Appreciation of the values involved in ensuring an alcoholic potential (Table 4) demonstrates the obtaining of wines of over 14 v.% alcohol, against the background of sufficient acidity.

CONCLUSIONS

The cultivars Cabernet cubin, Cabernet dorio and Cabernet dorsa have adapted well to the environmental conditions of southern Romania, obtaining good and superior quality productions, but at this stage (research so that it can later be transferred into their cultivation), it cannot be discussed which areas would be more suitable for them or where the possibility of obtaining wines could be appreciated from a qualitative aspect.

As a first step to follow would be to continue the research for several years and taking them into cultivation afterwards, in the beginning on smaller areas, so that later, the results will determine whether it is possible to move to significantly larger areas.

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ANALYSIS OF SOME BIOCHEMICAL COMPOUNDS INVOLVED IN ADAPTATION MECHANISMS OF VINE TO THE MINIMUM TEMPERATURES DURING THE DORMANT SEASON

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Abstract

The state of endodormancy in the vine as an adaptation reaction to unfavourable temperature conditions, has a complex character and is the result of multiple biochemical and physiological processes that take place at the level of the tissues of the vine shoots. In this study, the adaptation and response reactions of Merlot, Cabernet Sauvignon and Fetească neagră grapevine varieties to the temperatures during the dormant season in the Banu Mărăcine wine-growing centre are monitored, by following the evolution of some biochemical compounds involved in these mechanisms: evolution of free water (%), bound water (%), total water (%) and total dry matter (SUT %), as well as the evolution of carbohydrates (soluble sugar and starch) in annual and multiannual vine wood. A grouping of the analyzed varieties is made according to the storage potential of carbohydrates under the different conditions of minimum temperatures.

***Key words**: dorm eat season, grapevine varieties, biochemical compounds.*

INTRODUCTION

Establishing the relationship between the climatic resources, soil and the vine variety as well as the biochemical processes that take place under their influence, confirms the viticulture vocation of the studied areas as well as the recommendation of some varieties that are required for the production of wines for current consumption and for higher quality wines (Călugăr et al., 2009; Ciupureanu et al., 2016; Khan et al., 2022; Chou et al., 2023). The conditions of climate changes that are related in particular to water and thermal stress, require, in horticulture, a thorough knowledge of the variety that must be cultivated in certain cultivation areas (Dejeu et al., 2005; Cristescu, 2010; Hall and Jones, 2010; Jones and Alves, 2012; Climaco et al., 2012; Nicu and Mandă, 2012; Van Leeuwen and Darriet, 2016; Alikadic et al., 2019; Cichi et al., 2021; Costea and Capruciu, 2022). The quality of the grapes is also directly influenced by variety, ecoclimatic conditions of the applied agrotechnical works and zoning (Makra et al., 2009; Bunea et al, 2013).

The temperature of the environment, the light and the humidity are essential features of the climate in a certain wine-growing area, with a decisive impact on the development of the biological, physiological and biochemical processes of the vine (according to Cristescu et al., 2010; Jones et al., 2010; Costea et al., 2015; Cichi et al, 2016; Costea et al., 2021). Temperature is an important environmental factor affecting almost all aspects of growth and development in plants. The grapevine (Vitis spp.) is quite sensitive to extreme temperatures (Venios et al., 2020). The free water and bound water have a special role in the life of plants due to the relationships that exist between the forms of water and the various biochemical components in connection with the frost resistance process (Chirilei et al., 1970; Amira et al., 2010; Kopali et al., 2021). The transition of water from the free form to the bound form occurs gradually and is influenced by environmental conditions and plant metabolism (Rossouw et al., 2017, Junges et al., 2020). The dynamics of total water during the dormant season in the grapevine show differences depending on the genetic factors (variety), the metabolic particularities of the varieties during the acclimatization period (accumulation of osmotically active substances), climatic factors (minimum temperatures during winter) as well as of the climatic characteristic of the previous vegetation period (Fraga et al., 2014; Bucur și Babeș, 2016; Bernardo et al., 2018). The defence mechanism of the vines against the action of low temperatures has two essential sides that have the same goal: increasing and maintaining the water bound in the cells at the limit of gelation and decreasing the freezing point of the cellular solution (Camps & Ramos, 2012; Trudi et al., 2015). Carbohydrates, a group of substances of particular importance for vines, have been studied by various researchers. Since 1926, Alexandrov and Makarevskaia, cited by Stoev, 1979, found that in winter the roots of the vine contain rich reserves of starch and sugar and the starch fills all the tissues of the bark and wood, representing one of the first works on the dynamics of carbohydrates in vines shoots. These researches were continued by Costea et al., 2010; Ferrara et al., 2022. The better the maturation of the wood, the greater the reserves of substances accumulated in the wood, the plant being more resistant to low temperatures in winter (Dejeu, 2005). The data recorded during the research show that the ecological factors, namely the water regime during the vegetation period, the drop in temperature in the autumn-winter interval and the protection by burial, significantly change the water balance (total, free, bound water) in woods of different ages (according to Bertamini et al, 2006; Bucur and Dejeu, 2020). All of these factors influence directly the solubility and transport of synthesized organic substances (carbohydrates, amino acids, lipids, etc.), ensuring a way of permanent regulation of the metabolic processes of synthesis and hydrolysis, with consequences on the frost resistance potential of the grapevines (Zapata et al., 2004; Stroe and Bucur, 2012; Bucur and Babes, 2016; Medici et al., 2014; Burzo, 2015; Căpruciu, 2022). The frost resistance of the grapevine is a complex physiological-biochemical property influenced by a series of genetic, ecological, technological factors etc., which condition each other (Cichi, 2006; Burzo, 2015, Ribeiro et al., 2018). Also, the dormant period can be seen as a genetically controlled process, the expression of its genes manifesting under the action of environmental factors and especially of photoperiod and temperature (Rotaru et al., 2008; Torregrosa et al., 2017).

MATERIALS AND METHODS

Sampling Sites - The Banu Mărăcine viticultural center has a heterogeneous landform of hilly areas, consisting of wide valleys with an asymmetric profile and a variation of the topoclimate conditions, with moderate to strong slopes (6-32%) that reduce the force of air flows, ensuring a calm circulation of the air favouring the development of the biochemical and physiological processes of the grapevine. Located at 176 m altitude with 44º19' north latitude and 23º48' east longitude, Banu Mărăcine belongs to the A_3 oenoclimatic zone which includes viticultural centers producing mainly high-class red and aromatic wines and, secondarily, quality table wines (Teodorescu et et al., 1987). The climate is temperate-continental with Mediterranean influence, characterized by mild winters, hot summers with a high number of tropical days, maximum temperatures being above 30˚C. The experiments were organized within the Banu Mărăcine viticultural area in parcels placed in identical orographic conditions (plateau conditions) on a slightly levigated reddish brown soil. The varieties included in the study are grafted on Berlandieri x Riparia SO4 rootstock, with 2 x 1.2 m planting distance, semi-high growth with multiple Guyot cutting system, with a crop load of 40 buds/vine.

Biological material - The observations and determinations were conducted for the Merlot, Cabernet Sauvignon and Fetească neagră wine grape varieties during the 2020-2022 period within Banu Mărăcine - the Didactic Research Station of the University of Craiova. The determinations were done on annual and multiannual wood (3 years) collected at an interval of 10 days during the dormant period (XI-III), observing the dynamics of the main biochemical compounds (free water, bound water, total dry matter, soluble sugar, starch). In this sense, 10 canes/variety/type of wood were collected from 10 stumps from each variant. The analyzes were carried out in the viticulture laboratory of the Faculty of Horticulture, University of Craiova.

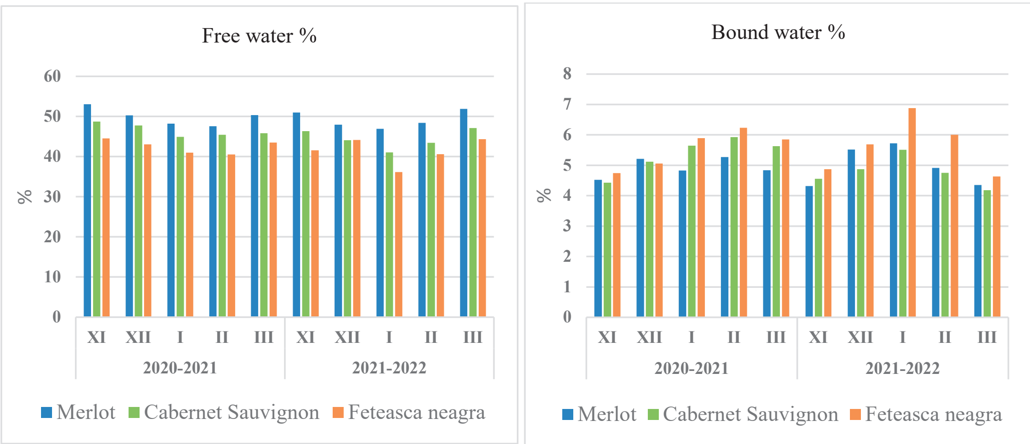
Methods - The monitoring of the minimum temperature during the dormant period was carried out at the Banu Mărăcine Meteorological Station. To determine the free water, consecutive weights were carried out on an analytical balance with a precision of 0.01 g until the difference between the weights did not exceed 0.03 g. In order to determine the bound water and the total dry matter, the oven - drying method was used (Căpruciu, 2016). The canes/woods were shredded and placed in an oven at a temperature of 105°C. The evolution of the water was observed after 48h. The obtained data were calculated with the formula: $U = m_1 - m_2 / m_1 \times 100$ (%), where, U = humidity, m_1 = sample mass before drying; m_2 = mass of the sample after drying. The total dry matter was calculated by the difference using the formula: SUT (%) = 100 - U (%). The carbohydrates were determined by spectrophotometric analysis. By this method, the extraction of soluble sugars is made with 80% volume alcohol solution, the starch with 52% volume perchloric acid solution and then the treatment is with 0.2% anthrone ($C_{14}H_{100}$) solution. The obtained colour intensity (with transparent blue-green colour shades) is measured colorimetrically, using UV-VIS Spectrophotometer at a wavelength of 620 nm (Comsa et al., 2013; Călugăr et al., 2010). The data resulting from the spectrophotometric reading were calculated as follows: Sugars = $E_c - E_a/E_b \times 50$; Starch = $E_d - E_a/E_b \times 50$; in which: E_a, E_b, E_c, E_d = extractions of solutions a, b, c, d; 50 = concentration of standard solution.

Statistical Analysis - In order to determine the content of biochemical compounds in the grapes of the Merlot, Cabernet Sauvignon and Fetească neagră varieties during the dormant season of 2020-2021 and 2021-2022 in the Banu Mărăcine viticultural center, the analysis of variance (ANOVA) was used. The differences between the means values of biochemical compounds were tested with the Duncan test (using the SPSS 16 program), the results being expressed as mean ± standard deviation (SD). Also, the coefficient of variation (CV %) was calculated.

RESULTS AND DISCUSSIONS

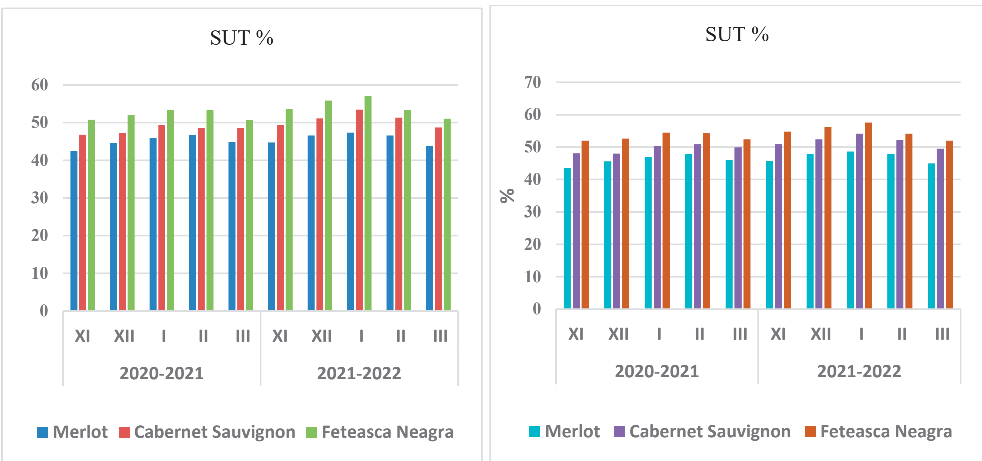
The biochemistry of plants can be directed towards the increasing resistance to adverse climatic conditions, the assessment of resistance can be defined with the help of biochemical and physiological indices such as water or carbohydrate content. The ratio between free and bound water is an essential indicator in determining the behaviour of vines at low temperatures during the dormant period (Gezici-Koç et al., 2017). In the woods of the Merlot variety, in the 2020-2021 period, the highest values were recorded in free water (53.03% in annual wood and 52.06% in multiannual wood in December 2021) with minimum values recorded in the 2021-2022 period of 46.91% in annual wood and 45.81% in multiannual wood within low temperatures in January (absolute minimum temperature of -10.5°C). In December, the lowest free water content was recorded in the Fetească neagra variety among the analysed varieties (44.5% in annual wood and 43.4% in multiannual wood in 2020). Chapman et al. recorded similar results regarding the water content of the canes/woods of the Cabernet Sauvignon variety in 2005 and Tomoiagă et al. for the Fetească neagră variety in 2020. The behaviour of the Cabernet Sauvignon variety at low temperatures was also studied by Adams in 2017. The water content of the Merlot variety was maximum in January with similar values for both the studied intervals as well as for the type of wood (5.8% in January 2021 in annual wood respectively 5.7% in January 2022 in annual wood and 5.5% bound water was recorded in multiannual wood) within absolute minimum temperatures of -10.5^{0}C. And Junges et al., 2020 analyse the content in biochemical compounds of the Merlot variety with similar results. Regarding the dynamics of the accumulation of bound water, it is found that in all the analysed varieties at the beginning of the dormant period the values are low, increasing with the decrease in temperatures, and decreasing again at the beginning of March. It is found in all the studied varieties that in multiannual vine wood both free and bound water have lower values compared to annual wood (Figure 1). Similar studies were conducted by Jităreanu et al. in 2011 in

Cotnari. Following the dynamics of SUT (%) in Figure 2, a maximum of accumulated dry matter can be observed in January 2022 in the Fetească neagră variety (57.03% in annual wood and 57.60% in multiannual wood) which coincides with a minimum of total water (42.9% in annual wood respectively 42.3% total water in multiannual wood). It is observed that the SUT (%) from the multiannual wood is higher than that recorded in the annual wood for all the analyzed varieties. The content in SUT (%) recorded during the dormant period of 2021-2022 in wood recorded higher amounts compared to the previous year (Figure 2). Analyzing the dynamics of carbohydrates in the Merlot variety, lower contents of soluble sugar can be noted in annual vine wood, with a maximum of 10.76% and in multiannual wood with 9.85% at the end of January (abs. min. temp. of -10.5^{0}C) corresponding to a minimum starch of 3.01% in annual vine wood and 3.25% in multiannual vine wood (January 23, 2021). The maximum average values were recorded in the Cabernet Sauvignon variety in both years, with higher values in January 2021 in annual wood (11.13% soluble sugar compared to 10.15% in January 2022). In multiannual wood, the soluble sugar content is lower (9.33%) with a minimum starch of 4.03% (January 2021), respectively 9.23% soluble sugar and 4.14% starch in January 2022 (Figure 3). The maximum values of soluble sugar were recorded in the annual wood of the Fetească neagră variety with 13.33% in January 2021 and 12.56% in January 2022. The values recorded in the multiannual wood are lower (10.12% and 11.33%). Regarding the starch content in the annual vine wood, the differences are significant with a maximum at the end of the dormant period of 7.21% in the annual wood of the Cabernet Sauvignon variety. Both in the annual and in the multiannual vine wood, a constant increase in starch can be found at the end of February and the beginning of March, in all the analyzed varieties (Figure 3). The quality of Fetească neagră and Merlot varieties depending on the cultivation area and climatic conditions was also studied by Onache et al in 2020, Trejo-Martínez together with the research team in 2009 as well as Bucur and Dejeu in 2020. In terms of the starch hydrolysis potential in the Banu Mărăcine viticultural center, the Fetească neagră variety stands out, followed by Cabernet Sauvignon and Merlot (Figure 3). Observing the dynamics of the evolution of carbohydrates in the annual and multiannual vine wood of the studied varieties during the dormant period, a quantitative increase in soluble sugar and a decrease in starch can be observed in all varieties, the differences being given by the low temperatures and the metabolic particularities of the varieties. In the cells of plant tissues, two types of water are highlighted: free and bound water. Furthermore, Khan et al. (2016) mention in plant-based food materials three types of water: intercellular, intracellular water and cell wall water. Free water, or capillary water, comes mainly from the intercellular space, while intracellular water and cell wall water is known as bound water, being osmotically or physically (Côme, 1992; Khan et al., 2016) linked to various substances with a protective role (proteins, soluble sugars, etc.). A series of researches have demonstrated that in freezing tolerant plants, ice formation is extracellular and results in the dehydration of living cells as intracellular water is drawn to extracellular ice masses (Levitt, 1980; Côme, 1992; Dereuddre et al., 1992). Based on the recorded results regarding the average content of total water (%), the free water and bound water during the two dormant seasons, it is observed that there are differences between the three varieties. Among the three varieties, Merlot has the highest content in total water and free water, but also the lowest content in bound water in both annual woody shoot and in multiannual wood (Table 1). Although there are differences between Fetească neagră and Cabernet Sauvignon regarding the types of water, they are statistically significant only for the total water content in both annual and multiannual wood ($p \leq .01$). Some studies show that the varieties that stood out through a higher tolerance to negative thermal stress during the winter have the ability to fix a greater amount of bound water in the cells of the canes/wood tissues (Cichi, 2005; Adams, 2017; Bernardo et al., 2018). Among the analyzed varieties, the highest content in bound water was recorded in the Fetească neagră variety, the differences being significant compared to the Merlot variety ($p \leq .05$). During the dormant season, the differences were highlighted between the three varieties regarding the content of some biochemical compounds with a protective role at the negative critical temperatures during winter (Table 2). The Feteasca neagra variety recorded the highest SUT content (%) both in annual woody shoot and in multiannual wood, the differences being significantly positive compared to Cabernet Sauvignon (Tukey HSD, $p \leq .05$) and Merlot ($p \leq .01$). Although there are differences between the three varieties regarding the content of soluble sugars, these are not statistically significant (Table 2). The differences are also observed in terms of starch content, the Merlot variety recording the lowest values both in the annual woody shoot and in the multiannual wood, the differences being significantly negative compared to the Feteasca neagra variety ($p \leq .05$).



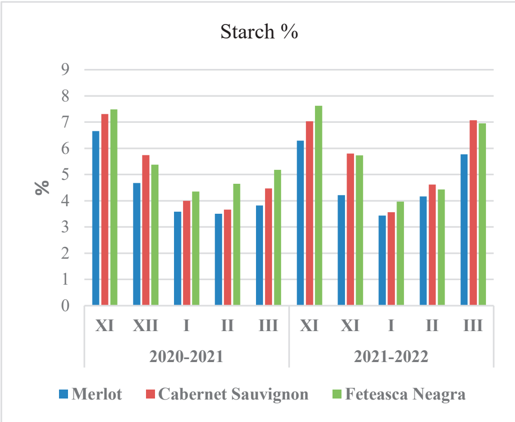
(a) (b)

Figure 1. The dynamics of free water (a) and bound water (b) content in annual vine wood during the dormant season

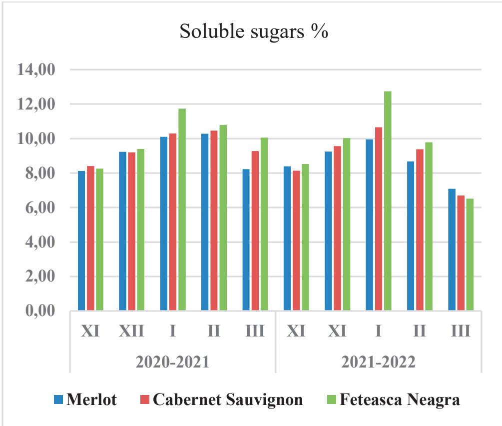


(a) (b)

Figure 2. The dynamics of SUT content (%) in annual (a) and multiannual vine wood (b) during the dormant season



(a)



(b)

Figure 3. The dynamics of the content of starch (a) and soluble sugars (b) in annual vine wood during the dormant season

Table 1. The content of types of the water during the dormant season*

Variety	Total water %		Bound water %		Free water %	
	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood
Merlot	54.65 ± 1.54^{a}	53.44 ± 1.61^{a}	4.79 ± 0.34^{b}	4.77 ± 0.29^{b}	49.55 ± 0.47^{b}	48.51 ± 0.46^{a}
Cabernet Sauvignon	50.52 ± 2.00^{b}	49.36 ± 1.93^{b}	5.06 ± 0.29^{ab}	4.91 ± 0.26^{ab}	45.47 ± 1.50^{ab}	44.49 ± 1.29^{bc}
Feteasca neagra	46.91 ± 2.11^{c}	45.91 ± 1.83^{c}	5.58 ± 0.13^{a}	5.44 ± 0.20^{a}	41.95 ± 0.82^{a}	41.07 ± 0.48^{c}

*Average of seasons 2020/2021 and 2021/2022/ Note: Means separation by HSD Tukey`s test at $p\leq0.05$. Means with the same superscript are not statistically significant

Table 2. The content of the main biochemical compounds during the dormant season*

Variety	SUT %		Soluble sugars %		Starch %	
	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood
Merlot	45.35 ± 1.54^{c}	46.54 ± 2.60^{c}	9.14 ± 1.12^{a}	8.60 ± 0.99^{a}	4.61 ± 1.26^{b}	6.49 ± 1.20^{b}
Cabernet Sauvignon	49.44 ± 2.01^{b}	49.65 ± 3.91^{b}	9.33 ± 1.54^{a}	8.77 ± 1.08^{a}	5.49 ± 1.78^{ab}	7.45 ± 1.46^{ab}
Feteasca neagra	53.09 ± 2.11^{a}	54.07 ± 2.86^{a}	10.12 ± 1.69^{a}	8.96 ± 1.47^{a}	5.76 ± 1.73^{a}	7.93 ± 1.25^{a}

*Average of seasons 2020/2021 and 2021/2022 / Note: Means separation by HSD Tukey`s test at $p\leq0.05$. Means with the same superscript are not statistically significant

CONCLUSIONS

It is observed in all the studied varieties, that both free and bound water are found in larger quantities in the annual wood compared to the multiannual wood. It is also found that the free form of water decreases progressively with the entry of the vine into the dormant period until February, after which it starts to grow again, the dynamics being different from one variety to another. Parallel to the decrease in the content of free water in all the studied varieties, an increase in the content of bound water is observed, with variable intensity depending on the variety and thermal conditions. During the dormant period in the studied varieties, it is observed that along with the decrease in the water content of the annual and multiannual wood, there is an increase in the content of total dry matter (SUT (%).

The dynamics of the content of starch and soluble sugars show differences depending on the variety and the thermal conditions from November to March, the most intense metabolic transformation of starch into soluble sugars being recorded in the months of January and February.

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AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF TABLE GRAPES VARIETIES, GROWN IN THE TEMPERATE-CONTINENTAL CLIMATE FROM SOUTHWESTERN ROMANIA

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Abstract

Ensuring a diverse range of grape varieties for fresh consumption, for as long as possible during a year, represents an equally important goal not only for producers but for traders as well. Our paper presents results regarding the evaluation of seven table grape varieties, six seeded varieties: 'Cardinal', 'Victoria', 'Muscat Hamburg', 'Alphonse Lavallée', 'Matilde', 'Black Magic' and one seedless variety, 'Attica', in specific pedo-climatic conditions from South-Western Romania. Observations, measurements and analyses were focused on: agrobiological, quantitative and qualitative characteristics (weight, length and width of bunch; berry weight, their length and width; grape yield, sugar content, titrable acidity, °Brix/acidity ratio). The assessment of vulnerability of table grape varieties at winter temperature conditions were also analysed. In the conditions of a low supply and high demand for table grapes at the end of July -middle of August in our country, the good productions and early harvesting of the 'Victoria', 'Black Magic' seeded varieties and 'Attica' seedless variety, represents a high market opportunity for Romanian table grape producers.

Key words*: ampelographic features, grape market, grapevine, seedless.*

INTRODUCTION

Grapes for fresh consumption are fruits appreciated by consumers all over the world for their sensory, nutritional attributes (Agulheiro-Santos et al., 2022 ; Premachandran A. et al., 2019 ; Soural et al., 2019), as well as their protective role against liver, cardiovascular diseases, etc. (Liang et al., 2016).

With a worldwide production of approx. 30.11 million tons (OIV, 2021a), a total volume of approx. 3.7 million tons of exports and 3.4 million tons of imports, table grapes are the most demanded fruit worldwide, after apples, oranges and tangerines/mandarines (USDA, https://apps.fas.usda.gov/psdonline/app/index .html#/app/home/statsByCountry). The EU is the fifth world producer (5.8% of world production), China still being the largest producer (45.96%), followed by India and Turkey (USDA, 2022).

When purchasing table grapes, consumers show the importance of the various attributes that grapes must have. Medium bunch size grapes (400-500 g), with large berries, with sweet-sour juicy and crunchy flesh, seeded or seedless (Costescu A., 2013) are preferred. The Spanish consumers gave the greatest importance to taste, sweetness, thin skin and the absence of seeds (Piva et al., 2006), while the Chinese consumers prefer the intense red to dark purple colour of the skin and the muscat flavour (Zhou et al., 2015). On the other hand, when promoting new varieties from the international or national assortment, table grape producers put their focus on making them competitive from the point of view of: availability on the market (extra-early and early varieties), increased yield, commercial characteristics and resistance to diseases, pests or unfavourable environmental factors.

The textural changes of grape berries during transport or storage are also important characteristics in quality assessment and marketability of table grapes (Ejsmentewicz et al., 2015; Stroe et al., 2022).

The cultivar, climate (Chedea et al., 2021) and the cultivation practices have a decisive role in ensuring sustainable and quality grape production. Among the cultural practices they are mentioned: conventional or greenhouse cultivation (Pisciotta A. et al, 2022); pruning and training system (Costea et al., 2017; Măracineanu et al., 2022); soil and water

management (Permanhani et al., 2016), desease and pest control (Maia et al., 2014); the use of chemical compounds and plant hormones (Koyama et al., 2019; Shahab et al., 2020) etc.
The areas cultivated with table grape varieties have recorded a continuous decline in the last three decades in Romania (Cichi et al., 2019). In 2021, the total area under production with table grapes represents only 3.8% of the total vine-growing area under production in Romania. Consequently, the production is also reduced, approx. 52 thousand tons (Romanian NSI), far below the requirements of consumers in our country (7.9 kg/capita), which makes imports in 2021 to represent approx. 53 thousand tons (https://www.oiv.int/what-we-do/data-discovery-report?oiv). Although Romania has valuable varieties (Bucur & Dejeu, 2018; Cichi et al., 2012; 2017; Stroe, 2016; 2021; Rotaru et al., 2010), the largest share in the culture is held by the varieties: ‘Chasselas doré’ (40%) , ‘Afuz Ali’ (25%) and ‘Muscat Hamburg’ (approx. 12%). The need to renew the range of table grapes according to consumer requirements and the extension of the harvest period, are therefore, important conditions for increasing competitiveness on the market for Romanian table grape producers. For this purpose, in the last period, various varieties from the international range were introduced in the south of Romania and some varieties were reconsidered by the producers, such as: ‘Cardinal’, ‘Victoria’, ‘Alphonse Lavallée’ and others. In this context, the aim of this study was to evaluate some agrobiological, productive and qualitative characteristics of seven varieties of table grapes in the specific conditions of the south-west of Romania. Among these, there are ‘Black Magic’, ‘Matilde’ and the ‘Attica’ seedless variety which, to our knowledge, has not been studied in our country until now.

MATERIALS AND METHODS

Location and climatic characteristics. The study was conducted for three consecutive years (2019-2021) in a table grape commercial vineyard, located in Plenita-Orodel (Plaiurile Drancei vineyard, Dolj County), in the south-west part of Romania. From a geographical point of view, the studied vineyard area is located between the parallels of 44°13'00" north latitude and 23°23'00" east longitude (cca.180 m elevation). The main climatic characteristics during the study period are shown in Table 1. The weather data were obtained from the Craiova meteorological station (44°13' latitude and 23°52' longitude, 192 m altitude, Dolj County). In terms of helio-thermal resources, the studied years were favourable for the table grapes growing (IH5) but, there is still a semi-arid aspect during the year and growing season, based on De Martonne Aridity Index (Table 1).

Plant material. Six seeded table grape varieties: ‘Cardinal’, ‘Victoria’, ‘Muscat Hamburg’, ‘Alphonse Lavallée’, ‘Matilde’, ‘Black Magic’ and one seedless variety, ‘Attica’ were used (Table 2). The study was conducted on 8-10 years old vines. The randomized experimental design was used. Ten vines per variety were selected for the study, in three replications. The vines were cultivated under the same growing conditions, using rootstock Berlandieri x Riparia SO_4, with the 2.5 x 1.0 m spaces, semi-tall shape of the stem (with a trunk of 0.8 m), Double Guyot pruned, 25 buds/vine (8-10 buds/cane and 2 buds/spur), without irrigation. The viticultural management (fertilizer application, pest, diseases and weed control, etc.) was applied for all varieties in the same way.

Agrobiological, quantitative and qualitative characteristics. The ampelographic parameters were defined and recorded in accordance with OIV standardized descriptors and methods (OIV, 2009a; OIV, 2021b; UPOV, 2008). Percentage of fertile shoots, absolute and relative fertility index (A.f.i., R.f.i.) were established and calculated according to Cichi et al., 2022.

Sampling measurement and analyses. 20 bunches for each variety, 35 berries from the middle part of bunches, in 3 replicates, were used for measurements and analyses of bunch and berry traits, at full maturity. The berry diameter was determined with a digital caliper and the mass of bunches and berries with an analytical balance. SSC (°Brix) was measured using Kruss Optronic Hand Refractometer Hrot 32. The TA was determined by the titration method, NaOH 0,1N until pH 7.0 and expressed in gL^{-1} tartaric acid. The SSC and TA were done in three replicates. The maturity index was calculated as ratio SSC/TA, for each sample.

Table 1. Main climatic indexes of the experimental site

Climatic Index	Average 2019-2021	Class
SAT (Sum of average daily temperature > 10°C, April 1st to September 30th)	3853	
Winkler Index	1921	Temperate
Huglin`s heliothermal index (IH)	2503	IH5-Warm
Annual Rainfall (mm)	515	Normal for region
Rainfall in the growing season (mm, April 1st to September 30th)	305	Normal for region
De Martonne Aridity Index (I_{DM}, year)	17.43	Semi-arid
De Martonne Aridity Index growing season (I_{DM}, April 1st to September 30th)	10.32	Semi-arid

Table 2. List of varieties included in the study

Variety	Variety number VIVC	Pedigree confirmed by molecular markers Prime name Parent 1 x Parent 2	Country of origin of the variety	Skin Berry color*
'Cardinal'	2091	Alphonse Lavalée x Reines des vignes	USA	Rg
'Victoria'	13031	Cardinal x Afuz Ali	Romania	B
'Muscat Hamburg'	8226	Schiava grossa x Muscat of Alexandria	UK	N
'Alphonse Lavalée'	349	Dodrelyabi x Muscat Hamburg	France	N
'Black Magic'	7569	Moldova x Marshalskii	Republic of Moldova	N
'Matilde'	7512	Italia x Cardinal	Italy	B
'Attica'	17309	Alphonse Lavalée x Black Monukka	Greece	N

*B-blanc; N-black; Rg- red. (Source: VIVC, https://www.vivc.de/)

Statistical analysis. Each variable was examined by analysis of variance (One-way ANOVA). The morphometric, biochemical and productive characteristics are presented as means and standard deviation of each variable. All variables that were significant in the F test were analysed by HSD Tukey's test to mean separation and to establish if there were significant difference.

RESULTS AND DISCUSSIONS

Cold resistance. The resistance of table grape varieties to the critical conditions of negative temperatures during the winter is a valuable traits (Lisek, J., 2014; Vujović et al., 2017), especially in temperate climates, where such temperatures periodically occur (Bucur & Dejeu, 2020; Rotaru et al., 2010). The resistance of the vine to frost is influenced by various factors, among which: the variety, the climatic conditions during the vegetation period, the cultivation practices, the cold acclimation, intensity of frost etc. which have an important role. In the experimental conditions, where the absolute minimum temperature (TNn) was -11.8°C, the percentage of viability of the buds was over 70% in all varieties, with no statistically significant differences between the varieties (Table 3).

Fertility. The grapevine fertility is a characteristic of each variety, but it can also be influenced by other factors, including: rootstock, climatic conditions during inflorescences differentiation in year n-1 and cultivation practices (Rives M., 2000). The shoot fertility in some cultivars and in certain viticultural areas may be the main productivity factor (Antcliff & Webster, 1955). On the other hand, fertility and its distribution along the length of the cane, condition the decisions regarding the pruning system for each variety (Olteanu et al., 2002). The 'Victoria' and 'Black Magic' varieties have the highest percentage of fertile shoots and A.f.i. (Table 3). The significant differences regarding the percentage of fertile shoots are also observed between 'Black Magic' compared to 'Matilde' ($p<0.05$) and 'Attica' ($p<0.01$) varieties. Also, Attica had the lowest values of the fertility indices. Similar results regarding the fertility of the 'Attica' variety were also reported by Mattheou et al., 1995.

Full maturity period. The earliness of production is an essential characteristic in the competitive table grape market, which decisively influences the income of the vine growers (Colapietra M., 2004). In the conditions of the temperate-continental climate in Romania, table grapes ripen between July 15 and October 30 (Olteanu et al., 2002). In the experimental conditions, full maturity was achieved starting from July 28 (‘Cardinal’ and ‘Black Magic’) until September 10 at the latest (‘Alphonse Lavallée’). The ‘Victoria’ variety, particularly appreciated for the precocity of ripening and the commercial value of the grapes (Gougoulias et al., 2015; Ferrara et al., 2017), reaches full maturity in the vine-growing areas in Romania starting from the 2nd-3rd decade of August (Bucur et al., 2018; Lepădatu et al., 1986). It should be noted that in the conditions of the south-west of Romania (Plenita vineyard), the Victoria variety makes good use of its precocity of full ripening, having an advance of at least one week compared to other cultivation areas in our country. During the study period, the ‘Attica’ seedless variety reached full maturity at the earliest on August 14 and at the latest on August 24, while the ‘Matilde’ variety reaches maturity at the earliest on August 25 (Table 3).

Table 3. The agrobiological and phenological characteristics (2019-2021)

Variety	Percentage of fertile shoots (%)	Bud viability*	A.f.i.	R.f.i	Full maturity Calendar
‘Cardinal’	$71.87{\pm}8.37^{cd}$	70.73±10.96	$1.59{\pm}0.22^{abc}$	$1.15{\pm}0.21^{a}$	28 Jul. - 02 Aug.
‘Victoria’	$87.49{\pm}7.39^{a}$	82.11±8.25	$1.73{\pm}0.17^{ab}$	$0.97{\pm}0.15^{ab}$	06- 09 Aug.
‘Muscat Hamburg’	$66.59{\pm}6.82^{cd}$	74.84±10.28	$1.30{\pm}0.16^{cd}$	$0.76{\pm}0.16^{b}$	25-30 Aug.
‘Alphonse Lavalée’	$68.63{\pm}6.78^{cd}$	75.13±11.84	$1.46{\pm}0.16^{bcd}$	$0.89{\pm}0.19^{ab}$	02-10 Sept.
‘Black Magic’	$84.11{\pm}7.20^{ab}$	77.41±11.89	$1.79{\pm}0.13^{a}$	$1.18{\pm}0.20^{a}$	28 Jul. – 02 Aug.
‘Matilde’	$73.84{\pm}7.71^{c}$	79.94±12.31	$1.43{\pm}0.17^{bcd}$	$0.80{\pm}0.15^{ab}$	25 Aug. – 04 Sep.
‘Attica’	$60.86{\pm}6.84^{de}$	$84.9{\pm}11.87^{NS}$	$1.25{\pm}0.12^{cd}$	$0.74{\pm}0.13^{b}$	14-24 Aug.

Note: Means±SD; Means separation by HSD Tukey`s test at p≤ 0.05; Means with the same superscript are not statistically significant; *at TNn = -11.8°C (dormant season 2018/2019)

Quantitative and qualitative characteristics. The size of bunches and berries is a valuable characteristic in the appreciation of table grapes, increasing their commercial value. ‘Attica’ and ‘Cardinal’ had the largest bunches as length, while ‘Victoria’ and ‘Matilde’ presented the largest bunches as width. However, a reduced variability of bunches length (CV%< 10%) can be observed in the ‘Black Magic’, ‘Matilde’ and ‘Attica’ (Table 4). ‘Victoria’ stood out with the longest berries, the differences being significant compared to the rest of the varieties (p<0.01). It can be noted that in all varieties the berries have low width variability (CV≤ 10%). In addition, the shape is typical for these varieties (OIV, 2009b, Colapietra M. et al., 2008), the berry length/width index being a specific characteristic of the variety.

Marketable fruit yield together with the moment of availability on the market, are key factors in terms of the profitability of a table grape variety (Zhou et al., 2015). The highest average yields per vine were recorded for the ‘Attica’ seedless variety (3.74 kg/vine), followed by the ‘Black Magic’ and ‘Victoria’ varieties (Table 5). There is a medium variability of the grape yield in the context of an important variability of bunches and berries weight (‘Muscat Hamburg’, ‘Matilde’ and ‘Alphonse Lavalée’). The marketable fruit yield variability can be explained, among others, by the fact that excesses of abundant precipitation were recorded in a short period of time (for example in June 2019-139 mm), making it difficult to control the diseases on the one hand and affecting pollination in some varieties on the other hand, with damages on quantitative and qualitative production. Another cause could even be the lack of irrigation, in the context of the semi-arid nature of the growing season.

Table 4. Morphometric characteristics of bunch and berry at full maturity (2019-2021)

Variety	Bunch length (mm)	CV%	Bunch Width (mm)	CV%	Berry length (mm)	CV%	Berry Width (mm)	CV%	Lenght/ Widht berry	CV%	Berry Shape
‘Cardinal’	227.75 ± 29.12^{a}	12.79	160.43 ± 12.33^{ab}	7.69	24.19 ± 2.07^{bc}	8.55	23.35 ± 2.01^{a}	8.61	1.04 ± 0.09^{d}	8.65	Globose
‘Victoria’	203.49 ± 21.44^{ab}	10.54	182.43 ± 27.93^{a}	15.30	27.51 ± 2.9^{a}	10.54	21.20 ± 1.26^{bc}	5.94	1.31 ± 0.13^{b}	9.92	Broad/narrow ellipsoid
‘Muscat Hamburg’	179.75 ± 20.80^{b}	11.57	149.19 ± 14.78^{ab}	9.91	18.72 ± 2.12^{e}	11.32	16.85 ± 1.37^{ef}	8.13	1.11 ± 0.10^{cd}	9.00	Globose/ovoid
‘Alphonse Lavalée’	153.18 ± 17.23^{bc}	11.25	136.56 ± 15.44^{b}	11.31	21.83 ± 2.65^{d}	12.13	21.45 ± 1.20^{b}	5.59	1.02 ± 0.12^{d}	11.76	Globose
‘Black Magic’	207.02 ± 13.20^{ab}	6.37	128.52 ± 14.26^{b}	11.09	24.9 ± 2.50^{bc}	10.04	17.66 ± 1.04^{de}	5.89	1.41 ± 0.15^{a}	10.63	Obtuse ovoid
‘Matilde’	212.25 ± 20.60^{ab}	9.70	170.27 ± 16.05^{ab}	9.43	24.12 ± 2.46^{bc}	10.20	20.13 ± 1.86^{c}	9.24	1.20 ± 0.12^{c}	10.00	Ovoid
‘Attica’	232.43 ± 18.28^{a}	7.86	169.33 ± 16.78^{ab}	9.91	25.31 ± 1.97^{b}	7.78	18.68 ± 1.35^{d}	7.23	1.36 ± 0.11^{ab}	8.08	Narrow ellipsoid

Note: Means±SD; Means separation by HSD Tukey`s test at $p\leq 0.05$; Means with the same superscript are not statistically significant.

Table 5. Quantitative characteristics of grape production at full maturity (2019-2021)

Variety	Bunch Weight (g)	CV%	Berry Weight (g)	CV%	Marketable fruit yield (kg/vine)	CV%
‘Cardinal’	398.92 ± 47.68^{abc}	11.95	8.49 ± 1.21^{a}	14.25	2.64 ± 0.17^{c}	6.43
‘Victoria’	439.44 ± 45.58^{abc}	10.37	8.21 ± 1.07^{ab}	13.03	3.13 ± 0.22^{abc}	7.03
‘Muscat Hamburg’	267.05 ± 48.53^{bcd}	18.17	4.03 ± 0.49^{f}	12.16	2.99 ± 0.40^{bc}	13.38
‘Alphonse Lavalée’	324.77 ± 54.93^{bc}	16.91	7.59 ± 1.05^{bc}	13.83	2.88 ± 0.42^{bc}	14.58
‘Black Magic’	365.04 ± 48.45^{abc}	13.27	5.61 ± 0.58^{d}	10.34	3.44 ± 0.26^{ab}	7.56
‘Matilde’	494.59 ± 93.40^{a}	18.88	6.82 ± 1.02^{bc}	14.95	2.65 ± 0.42^{c}	15.85
‘Attica’	465.15 ± 40.95^{a}	8.80	4.81 ± 0.36^{e}	7.48	3.74 ± 0.31^{a}	8.29

Note: Means±SD; Means separation by HSD Tukey`s test at $p\leq 0.05$; Means with the same superscript are not statistically significant.

Ensuring water through irrigation, especially for table grapes, is a way to counteract the effects of drought and ensure regular production (Satisha et al., 2006), various studies using yield and quality of grape production as indicators to characterize the reaction of different cultivars in drought conditions (Chaves et al., 2007). Studying the effect of a three variant pruning used for 'Black Magic' variety, in Boznia Herzegovina (3 x 1.2 m vine spacing, Moser cordon training system), Delic et al. (2017) concluded that the best results of the production (7.73-12.38 kg/vine) and fertility were obtained for the 40 buds/vine. Similar results were found when analyzing the positive influence of growth for load of buds/ vine on the productivity of the Black Magic in Republic of Moldova by Godoroja et al. (2020).

According to the commercial quality standards developed by the Working Party on Agricultural Quality Standards of the United Nations Economic Commission for Europe (UNECE, 2017) and OIV Resolution VITI 1/2008 (OIV, 2008), in order to satisfy the maturity requirements, the grape must have obtained a refractometric index of at least 16°Brix. For white/rose table grape varieties, fruits with a refractometry index lower than 16°Brix are accepted, provided that the sugar (expressed in g/L soluble sugars)/acid (expressed in gL^{-1} tartaric acid) ratio is at least equal to 20/1 if the Brix level is greater than or equal to 12.5° and lower than 14° Brix. For red colour table grape varieties, the grapes with a Brix degree equal to or above 12.5 and below 16° Brix, must present a minimum sugar/acid ratio of 20/1 to be considered as ripe (OIV, 2008).

The highest SSC content was recorded in the 'Matilde' and 'Muscat Hamburg', the differences being significant compared to the early ripening varieties. Among the early ripening varieties, 'Black Magic' recorded the highest sugar content and a balanced SSC/TA ratio, however, the differences are not statistically significant compared to 'Cardinal' and 'Victoria' (Table 6). The 'Attica' seedless variety recorded an average SSC of 17.03° Brix in the context of an AT of 5.62 gL^{-1} tartaric acid (Table 6).

It can be appreciated that all the varieties have ensured a balanced taste given by the SSC/TA, the maturity index being in agreement with the quality standards regarding the maturity requirements of the table grape varieties sold in the EU.

Table 6. Biochemical characteristics of grapes (2019-2021)

Variety	SSC °Brix	AT gL^{-1} tartaric acid	Maturity Index SSC/TA
'Cardinal'	14.11 ± 0.88^{bcd}	4.60 ± 0.36^{cd}	28.57 ± 2.24^{ab}
'Victoria'	15.26 ± 0.93^{bc}	4.96 ± 0.62^{c}	31.23 ± 3.82^{ab}
'Muscat Hamburg'	17.70 ± 0.86^{a}	6.08 ± 0.38^{ab}	32.25 ± 2.86^{ab}
'Alphonse Lavalée'	15.81 ± 1.04^{abcd}	6.65 ± 0.64^{a}	24.35 ± 3.60^{b}
'Black Magic'	15.47 ± 1.16^{bc}	5.10 ± 0.41^{c}	33.38 ± 6.43^{ab}
'Matilde'	17.85 ± 1.13^{a}	4.82 ± 0.39^{c}	37.53 ± 4.96^{a}
'Attica'	17.03 ± 0.94^{abc}	5.62 ± 0.56^{bc}	31.61 ± 3.68^{ab}

Note: Means±SD; Means separation by HSD Tukey`s test at $p\leq 0.05$;
Means with the same superscript are not statistically significant.

CONCLUSIONS

The varietal renovation and the promotion on the market of new table grape varieties (seeded and seedless), especially with early and late harvest, good yield, which ensures the staggered consumption over the longest period of time, are essential for increasing the competitiveness and efficiency of Romanian producers and simultaneously, ensuring consumer satisfaction. In the conditions of a low supply and high demand for table grapes at the end of July -middle of August in our country, the good productions, early ripening and availability on the market of the 'Victoria', 'Black Magic' seeded varieties and 'Attica' seedless variety, represents a high market opportunity for Romanian table grape producers.

There are improvements to be made for producing a good yield of high-quality fruit in these varieties, therefore, we consider it is necessary to continue the research in order to achieve a balance between vegetative growth and fruiting through appropriate cultivation practices (optimal load of buds/vines, number of buds/cane, number of inflorescences/vine, summer pruning, irrigation, control of diseases and pests etc.).

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IMPROVING SOME STEPS OF GRAPEVINE GROWING TECHNOLOGIES TO REDUCE PRODUCTION COSTS

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Abstract

Grapevine growing technologies require continuous adjustments to both climate instabilities and, in particular, current viticulture issues such as a sharp increase in prices for the vast majority of inputs and an increasing difficulty in finding labour at reasonable prices. The research focuses on a varietal assortment of five cultivars, as well as the main technological sequences of grapevine growing that require a significant amount of manual labour or inputs, such as soil management and summer pruning and activities. The influence of the experimental variants both on grape production and especially on the economic indicators was monitored. For each technological sequence, several experimental variants with different complexity were tried, adaptable to each vineyard with various technological and financial possibilities. In these circumstances, Romanian vineyards must align their organization, management, and growing technologies in order to compete with products from other countries for quality and price. To achieve this goal, in addition to ongoing organization and documentation, it is necessary to select technological options that allow for high-tech mechanization which is critical for lowering costs while still maintaining grape quality and lowering carbon emissions.

Key words*: grapevine, grapes, wines, quality, profit.*

INTRODUCTION

Grapevine growing technologies changed and improved continuously over time, especially as they became more intensive (Delrot et al., 2020). Today, grapevine growing technologies must be updated to reflect the current issues facing humanity in general and viticulture in particular, such as the difficulty to find qualified manual labour, the excessive increase in the price of most inputs due to the energy crisis, the urgent need to reduce soil and environmental pollution, and to decrease carbon emissions (Campbell, 2019). Last but not least, winemakers must contend with the extraordinary competition on the wine market, which sometimes exceeds legal boundaries (Wilson & Winchester, 2019). Soil management is a major component of production costs, with significant implications for grape production, soil quality, and environment sustainability (Lazcano et al., 2020). It is impossible to develop a technology framework with broad application due to the large climate variability, soil, and technological diversity in which grapevine is grown (Biasi et al., 2019). Growing technologies must be applied differently in accordance with the climate and economic realities, as well as the challenges in finding qualified labour (Chedea et al., 2021; Cichi et al., 2021). Furthermore, recently were recorded unprecedented price increases for the large majority of inputs, particularly fuels, fertilizers, and fungicides (Cataldo et al., 2021). All of these factors have determined major changes in both Romanian and global viticulture technology (Rahoveanu, 2021). The new technologies emphasis mechanization to counterbalance as much as possible for the shortage of manual labour and higher costs, a reasonable, balanced use of pesticides and fertilizers, carbon emissions mitigation by reducing soil and environmental pollution, correlated with high quality, healthy, and pesticide-free grapevine by-products obtained at a lower cost to be more competitive on the challenging international wine market (Clemens et al., 2018; Sun et al., 2022). In addition to the previously mentioned barriers, there is also the challenge of joining and, more importantly, surviving on a competitive wine market that has experienced powerful competition from both new world of wine as well as from European countries with tradition in viticulture, which offer high-quality wine by-products, sometimes at unbelievably low prices (Durmaz et al., 2019).

MATERIALS AND METHODS

The research was carried on in a young vineyard at the beginning of the full maturation in the Buziaș-Silagiu Viticultural Center from western Romania, during the growing season 2021 (typically favourable year for grapevine growing). In order to identify the most feasible solution in the existing environment which is characterized by the necessity to decrease carbon emissions, the overstated price increases for most inputs, and the increasing difficulty in finding labour, the improvement of the main technological sequences in grapevine growing technology was studied. The vineyard where the research was carried out is situated on a southern-exposed plot with a slope of 5-7%; planting distances were 2.2 m between rows and 1 m between vines per row, with a density of 4545 plants per hectare. The vines were trained as simple Guyot, with one or two one-year-old canes. The experimental variants for vineyard soil management were: S1 - complex variant (autumn and spring ploughing, 3 mechanical harrows per row, 3 mechanical harrows between rows); S2 - medium complexity (autumn ploughing, 2 mechanical harrows on row, 2 mechanical harrows inter-rows; S3 (Control) - lower complexity (autumn ploughing, rotary tillage and weeding); S4 - minimum complexity (two mowings of the cover crop and one ploughing every 2 years). The experimental variants for the improvement of pruning and training during the growing season were: P1 - complex (shoot thinning, tying, desuckering, and shoot trimming); P2 (Control) - medium complexity (shoot thinning, desuckering, and shoot trimming); P3 - reduced complexity (two mechanical trimming); and P4 - minimal complexity (one mechanical trimming). Five wine grape varieties - Cabernet Sauvignon, Feteasca neagra, Merlot, Feteasca regala, and Italian Riesling - with diverse biological characteristics and growth rates were the subjects of the research. The production obtained and the key economic indicators were observed and determined in experimental variants (production expenses, expenses for the experimental variant, cost price, grape production value and profit). Each experimental variant had four rows of vines. The samples for observations and analyses were collected from the rows in the middle of the experimental block. The vine row was approximately 250 meters long and divided into three plots, each with 20 vines (three replicates). The control variant in both experiments was chosen as the standard technological variant used in the plantation. It was analyzed how yields, costs, and economic indicators changed during the growing season 2021. All data on variable costs and expenses for cultivation operations were collected. Statistical analyses were used to clarify the research results and also to determine the correlations and regressions for economic indicators. A factorial analysis of variance and the Tukey test were used with Microsoft Excel, version 16.18, (180903) 2019, to compare the factor levels at significance $p < 0.05$. Averages per treatment were calculated for all parameters measured and used in statistical analyses. To summarize the main features of data distribution, descriptive statistics were computed for the variables under study.

RESULTS AND DISCUSSIONS

The soil maintenance system is an important technological step that has a significant impact on production, quality, carbon emissions, and last but not least, the major economic indicators. The need to decrease costs without compromising the quantitative and qualitative production parameters, the physical and chemical characteristics of the soil, or reducing carbon emissions to the minimum level has forced soil management to undergo continuous change. An important technological sequence, with major influence on production, quality, and carbon emissions and last but not least on the main economic indicators, is represented by the soil maintenance system. In last decades, soil management is in a continuous change and search, for the optimal option, imposed by the current needs to reduce costs, without affecting the quantitative and qualitative parameters of grape production, the physical-chemical properties of the soil, and to decrease to the minimum possible the carbon emissions.

Table 1. Soil management influence on grape production during 2021 growing season

Indicator	Variety	Experimental variants			
		S1 (± SD)	S2 (± SD)	S3 (Control) (± SD)	S4 (± SD)
Grape production (kg/ha)	Cabernet Sauvignon	10775±441	10345±506	9745±426	9220±411
	Feteasca neagra	9987±412	9526±415	8941±321	8383±305
	Merlot	9534±409	9105±398	8618±304	7815±296
	Feteasca regala	12575±582	12324±572	11765±527	10912±421
	Italian Riesling	11961±534	11683±514	10984±452	10268±398
Difference from control (kg/ha)	Cabernet Sauvignon	1030±93**	600±41*	-	-525±35*
	Feteasca neagra	1046±81**	585±37*	-	-558±33*
	Merlot	916±73**	487±29	-	-803±62**
	Feteasca regala	810±61*	559±38ns	-	-853±69*
	Italian Riesling	977±76**	699±49*	-	-716±51*

(ns, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001)

In order to save fuel and minimize greenhouse gas emissions, many vineyards have reduced soil tillage with agricultural machinery.

The reduction of soil loosening works to a minimum in the fourth variant also resulted in the lowest productions, with differences depending on the variety, ranging from 853 kg per hectare for the Feteasca regala variety to 525 kg per hectare for the Cabernet Sauvignon variety (Table1). The S1 with the most soil tillage works produced the highest yields; differences from the control fluctuated depending on the variety, ranging from 810 kg per hectare for the Feteasca regala variety to 1046 kg per hectare for the Feteasca neagra variety. All varieties reacted by reducing their output in direct correlation with the reduction of soil works (most of the differences were statistically significant).

In all varieties, the control variant produced reasonable grape yields by applying a minimum number of soil tillage; therefore it was a balance option between carbon emissions as low as possible and an acceptable production of grapes.

Table 2. Influence of vineyard soil management on economic indicators during 2021 growing season

Economic indicator	**Variety**	**Variants of vineyard soil management**			
		S1	**S2**	**S3 (Control)**	**S4**
Total production cost (euro/ha)	Cabernet Sauvignon	2720	2638	2494	2440
	Feteasca neagra	2835	2753	2609	2555
	Merlot	2560	2478	2334	2280
	Feteasca regala	2680	2598	2454	2400
	Italian Riesling	2590	2508	2364	2310
Costs with soil management variants (euro/ha)	Cabernet Sauvignon	462	380	236	182
	Feteasca neagra	462	380	236	182
	Merlot	462	380	236	182
	Feteasca regala	462	380	236	182
	Italian Riesling	462	380	236	182
Grape production value (euro/ha)	Cabernet Sauvignon	6530	6270	5906	5588
	Feteasca neagra	6053	5773	5419	5081
	Merlot	5778	5518	5223	4736
	Feteasca regala	5588	5447	5229	4850
	Italian Riesling	5316	5192	4882	4576
Cost price (euro/ton)	Cabernet Sauvignon	252	255	256	265
	Feteasca neagra	284	289	292	305
	Merlot	268	272	271	292
	Feteasca regala	213	211	208	220
	Italian Riesling	216	215	215	225

Gross profit (euro/ha)	Cabernet Sauvignon	3810	3632	3412	3148
	Feteasca neagra	3218	3020	2810	2526
	Merlot	3218	3040	2889	2456
	Feteasca regala	2908	2849	2775	2450
	Italian Riesling	2726	2684	2518	2266

However, the minimally invasive technologies cannot compete with the intensive variants regarding the level of grape production.

Different subsidies are imposed to compensate farmers for lower grape production in order to practise less polluting viticulture, because the higher price for organic products is not easily accepted by consumers on the Romanian market.

The analysis of the influence of soil maintenance variants on economic indicators (Table 2) produced distinct findings for each indicator. Obviously, less tillage decreased production costs. Therefore, the variant with the least tillage (S4) in all varieties resulted in a 300-euro-per-hectare decrease in grape production costs. The control variant also had a reasonable level of production costs for soil works, which were decreased by more than 200 Euros per hectare when compared to the most complex S1 variant. For soil management, the expenses with the experimental variant ranged from 462 Euros per hectare for the S1 to 182 Euros per hectare for the S4.

As the number of soil works decreased, the cost per tonne of grapes increased proportionally. The difference in grape production influenced the cost price to decrease, not the total cost of grape production. Although there were significant differences in grape production between the complex variant S1 and the less intensive variants S2 and S3, Feteasca Regala was the only variety where less tillage resulted in lower costs. The profit was correlated with the complexity of the soil works, and the level of production achieved in the intensive variants had an impact on the profit.

According to similar studies, Akdemir (2022) found that the experimental variants where soil tillage was done by machines recorded the highest values of profit per hectare. Also, Borca et al. (2020) found that less vineyard floor management resulted in lower production costs for six wine grape varieties in the Silagiu vineyards. Nan et al. (2021) validated the evidence, that complex soil works generate a higher profit, by showing that soil management through tillage and herbicides yielded a higher income than bare soil, in two wine grape varieties, Chardonnay and Fetească neagră, respectively.

Summer canopy management is another technological sequence that has significantly changed in recent years, due mainly to the manual labour availability (Bucur, 2021); depending on the technological option chosen, this sequence has a significant impact on grape production. Although the P1 - the variant with many summer canopy management interventions, some of them entirely manual - provided the highest grape production for all varieties, it is now used less frequently in vineyards because it is nearly impossible to ensure the necessary workforce, particularly in large vineyards (Table 3).

Tabel 3. Canopy management influence on grape production during 2021 growing season

Variable	Variety	Experimental variant			
		P1 (±SD)	P2 (Control) (±SD)	P3 (±SD)	P4 (±SD)
Grape production (kg/ha)	Cabernet Sauvignon	11256±527	10930±438	10456±399	9625±436
	Feteasca neagra	10653±452	10124±412	9211±418	8850±316
	Merlot	9783±411	9308±403	8724±335	8115±299
	Feteasca regala	12743±563	12491±579	11935±527	11317±504
	Italian Riesling	11992±519	11691±513	10815±449	10393±438
Difference from the control (kg/ha)	Cabernet Sauvignon	326 ± 18^{ns}	-	-474 ± 28^{ns}	$-1305\pm91^{***}$
	Feteasca neagra	$529\pm37^{*}$	-	$-913\pm71^{**}$	$-1274\pm97^{***}$
	Merlot	$479\pm28^{*}$	-	$-580\pm40^{*}$	$-1189\pm83^{**}$
	Feteasca regala	252 ± 11^{ns}	-	-556 ± 31^{ns}	$-1174\pm76^{**}$
	Italian Riesling	301 ± 14^{ns}	-	$-876\pm59^{*}$	$-1298\pm81^{**}$

(ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

The P2 (control variant) is ideal for small vineyards that cannot afford to invest in mechanical canopy management equipment. Summer canopy management was reduced by half in P2 compared to P1; grape production was satisfactory even though it was declining, with differences between 251 kg on hectare for the Feteasca regala variety and 529 kg on hectare for the Feteasca neagra variety. The lowest productions for all varieties were found in the P3, with two mechanical trimming, and P4 with one mechanical trimming for canopy management.

The P3 variant, however, can be a viable alternative to most varieties, with production differences of several hundred kilograms recorded, compared to the control. In the Cabernet Sauvignon, Fetească Regală, and Italian Riesling varieties, summer canopy management interventions (P1) did not result in significant increases in production to justify their costs. However, mechanized pruning had a significant negative impact on grape production for the majority of varieties.

During summer canopy management, the variants were classified similarly to those for soil management in order to evaluate economic indicators (Table 4). The mechanical canopy management reduces expenses significantly for the P3 and especially for the P4. In Feteasca neagra, for instance, expenses for canopy management, decreased from 325 Euros on hectare in the P1 to 160 Euros in the P3, and 80 Euros in the P4. In the summer canopy management, the level of production had a higher impact on the cost price and profit than did the level of expenses. Because the quality of mechanized works is still lower than that of manual ones, it is necessary to continually improve the machines used to optimize these activities (Wang et al., 2019; Gil et al., 2022). Scheduling the canopy management over an extended period of time (May-September), an interval that overlaps with holidays as well as the summer heat, makes it impossible to provide enough manual labour, especially in the current economic and social context (Somkuwar et al., 2019). For these reasons, the majority of vineyards are forced to carry out mechanized canopy trimming, which requires the purchase of special machines and equipment whose prices have recently increased significantly (Kurtural et al., 2021).

Table 4. Influence of canopy management on economic indicators during 2021 growing season

Economic indicators	**Variant**	**Variants for canopy management**			
		P1	**P2 (Control)**	**P3**	**P4**
Total production cost (euro/ha)	Cabernet Sauvignon	2643	2603	2483	2403
	Feteasca neagra	2763	2718	2598	2518
	Merlot	2478	2438	2323	2243
	Feteasca regala	2593	2553	2443	2363
	Italian Riesling	2508	2468	2353	2273
Costs with canopy management variants (euro/ha)	Cabernet Sauvignon	320	280	160	80
	Feteasca neagra	325	280	160	80
	Merlot	315	275	160	80
	Feteasca regala	310	270	160	80
	Italian Riesling	315	275	160	80
Grape production value (euro/ha)	Cabernet Sauvignon	6822	6624	6337	5833
	Feteasca neagra	6456	6136	5582	5364
	Merlot	5929	5641	5287	4918
	Feteasca regala	5663	5552	5304	5030
	Italian Riesling	5330	5196	4805	4619
Cost price (euro/ton)	Cabernet Sauvignon	235	238	237	249
	Feteasca neagra	259	268	282	284
	Merlot	251	262	266	276
	Feteasca regala	203	204	205	209
	Italian Riesling	209	211	217	219
Gross profit (euro/ha)	Cabernet Sauvignon	4179	4021	3854	3430
	Feteasca neagra	3693	3418	2984	2846
	Merlot	3451	3203	2964	2675
	Feteasca regala	3070	2999	2861	2667
	Italian Riesling	2822	2728	2452	2346

Schütte et al. (2020) investigated the cost distribution in grape production and discovered that labour accounts around half of the winery budget, followed by machinery costs and closely followed by chemicals, and this can be attributed to higher quality grapes that involve more pruning and canopy management.
Figure 1 shows the different grape production of the five varieties based on tillage and canopy management. Overall, the Feteasca regala yielded the most grapes, followed by Italian Riesling. Regardless of soil or canopy management, the Merlot variety produced the fewest grapes, while the Feteasca neagra variety produced slightly more. Of the four varietals, Cabernet Sauvignon had the most balanced grape production.

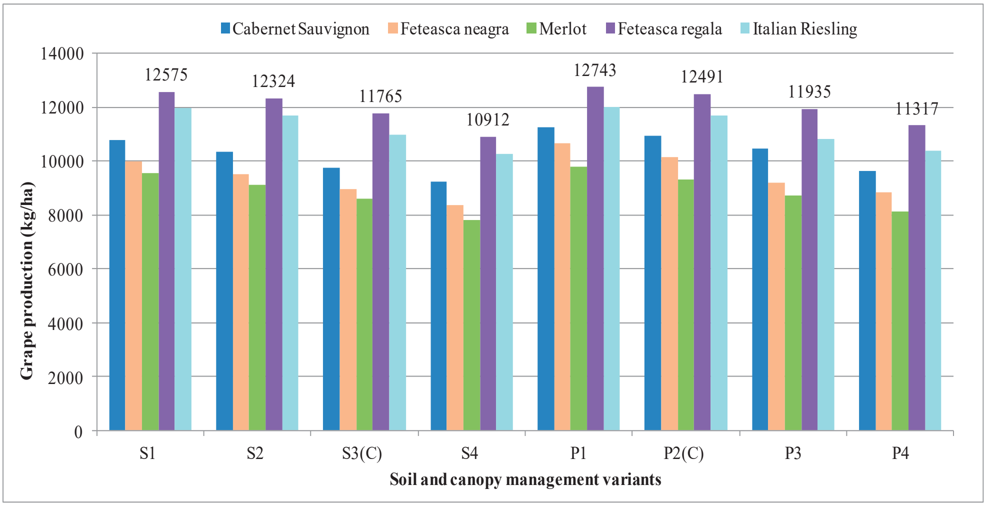

Figure 1. Soil and canopy management influence on grape production during 2021 growing season

Compared to the other soil and canopy management variants, the variant with two mowings of the cover crop and one ploughing every two years (S4), and one mechanical trimming (P4) had the lowest grape production. The highest grape yields were obtained when the soil was ploughed in the autumn and spring, supplemented with three mechanical harrows per row and three mechanical harrows between rows, correlated with canopy management through shoot thinning, tying, desuckering, and shoot trimming. Zumkeller et al. (2022) investigated the effect of reduced tillage and cover crops and discovered that, contrary to the presented results, the productive response of the vine and the grape production remains unclear.

CONCLUSIONS

For all grape varieties investigated, grape yield was higher in experimental variants with intensive soil tillage compared to minimal invasive soil management. Minimally invasive soil management options would undoubtedly require reimbursements to compensate for the difference in profit and to encourage winegrowers to choose friendlier, less invasive technologies despite the obvious decline in grape production. Among the investigated varieties, Feteasca regala proved to be the least sensitive to soil maintenance options. Within this variety, the complex variant of soil maintenance is not justified, because the production difference between it and the control variant S3 is small. The S2 option, with a favourable expense-to-production ratio, is the most commonly recommended option within this range.
In the Cabernet Sauvignon and Feteasca neagra varieties, the variant with minimal tillage (S4) had the smallest differences from the control (S3). This option remains a viable alternative, applicable only if the vineyard doesn't have the financial resources to pursue a more expensive option. The economic indicators were affected

differently by the soil maintenance options. S2 and S3 for the Riesling Italian variety were the only more affordable variants that recorded a lower price.

Profit is the most important economic indicator, and it has grown exponentially as soil maintenance options in all varieties have become more complex. Feteasca Regala and Riesling Italian varieties adapted best to lower-cost soil maintenance, with smaller profit differences between the complex variant (S1) and the other variants.

The summer canopy management represent another technological sequence that requires an upgrade, imposed by the difficulty in finding available manual labour during the growing season and less due to the invasive aspect. Therefore, the only currently viable option for the canopy management, particularly in large vineyards, is mechanization. But, even though the level of production expenses is significantly reduced by mechanization, all of the researched varieties experience a decline in profit as a result of the grape production decline.

Only the Feteasca neagra and Merlot varieties justify the complex variant (P1) for canopy management. In the Feteasca regala variety, the P3 variant, which consisted of two mechanized interventions on the canopy, was a cost-effective and viable option. With the exception of canopy management costs, a single mechanized intervention (P4) proved to be inefficient for all economic indicators studied, in all varieties.

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CLIMATIC CONDITIONS - IMPORTANT FACTOR OF THE GRAPES AND WINE TERROIR

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Abstract

The influence of terroir on the growth, development and productivity of the vineyard has been known since ancient times, but due to the increasingly changing climate, it is becoming an increasingly indispensable subject to study. The European continent remains to be affected by climate change, and the countries in the south-eastern part the most, or this was mentioned in the report `Climate change, impacts and vulnerability in Europe 2020`. The frequency and intensity of climate changes will impose the need to adapt technologies, the diversification of vine varieties or the adaptation of existing ones. The average air temperature on the territory of the Republic of Moldova registers an average increase of approximately 0.01°C/year during the period 1887-2010. Recent years have also seen an increase in temperatures of over 1°C compared to 10-15 years ago. The amount of water coming from the precipitation is less and less, and reaches the lower limit of non-irrigated vine cultivation.

Key words*: Climate change, Grapes, Terroir, Viticulture, Wine.*

INTRODUCTION

Climate change affects the entire agricultural sector, having consequences on food security and the economy of countries in vulnerable regions, including the Republic of Moldova. Climate change does not avoid the vine culture either, which reacts significantly to it.

The climate and climatic changes are included in the category of risk factors that contribute to the development of agro-food enterprises, a fact confirmed in various studies (Godoroja et al., 2022; Godoroja et al., 2023; Midari et al., 2022; Mogildea, 2023; Mogildea et al., 2023; Nicolaescu et al., 2023; Nicolaescu et al., 2022).

The final quality of grapes and wine, respectively, depends on several factors. We can talk about the grape variety, the type of soil, the cultivation technique but also the climatic conditions. The last one is a very important factor. Alternating periods of water stress with sudden changes in temperature have a negative influence on the health of the barrels and subsequently of the wine.

Plants that are more vulnerable during these times will be more fragile and more susceptible to disease. Some pests that are not present in certain areas will migrate and be potential vectors of viruses on crops.

The purpose of the research reflected in this article is to study the role of climatic conditions and their changes through the prism of the development of the wine sector.

MATERIALS AND METHODS

For the study of this article, the following databases and information were used:

- Data from local meteorological stations;
- National Bureau of Statistics (NBS) of the Republic of Moldova;
- Ministry of Agriculture and Food Industry of the Republic of Moldova;
- Food and Agriculture Organization of the United Nations (FAO Faostat);
- International Organisation of Vine and Wine (OIV);
- Official documents and special literature etc.

MS Office Excel (2019) was used for the mathematical processing of the data.

RESULTS AND DISCUSSIONS

During the analysed period, 2017-2022, in the Central part (Codru Region) of the Republic of Moldova the average annual temperature was

11.6^{0}C. The minimum average temperature for this period was 10.6^{0}C for 2021, and the maximum 12.7^{0}C for 2020.

In the period, 2017-2022, the average temperature of January was -0.95^{0}C. The average minimum temperature for this period was -4.2^{0}C for 2017, and the maximum 1.5^{0}C for 2020. The average temperature of February was 1.37^{0}C. The average minimum temperature for this period was -1.3^{0}C for 2018, and the maximum 4.4°C for 2020. The average temperature of March was 5.32^{0}C. The minimum average temperature for this period was 0.8^{0}C for 2018, and the maximum 8.4^{0}C for 2020. The average temperature of April was 10.98^{0}C. The average minimum temperature for this period was 8.5^{0}C for 2021, and the maximum 15.1^{0}C for 2018. The average temperature of May was 16.55°C. The average minimum temperature for this period was 14.4^{0}C for 2020, and the maximum 19.4^{0}C for 2018. The average temperature of June was 21.83^{0}C. The minimum average temperature for this period was 20.2^{0}C for 2021, and the maximum 23.5^{0}C for 2019. The average temperature of July was 23.0^{0}C. The average minimum temperature for this period was 22.1^{0}C for 2019, and the maximum 24.0^{0}C for 2021. The average temperature of August was 23.58^{0}C. The average minimum temperature for this period was 21.7^{0}C for the year 2021, and the maximum 24.6^{0}C for the year 2018. The average temperature of September was 17.95^{0}C. The minimum average temperature for this period was 15.7°C for 2021, and the maximum 20.8^{0}C for 2020. The average temperature of October was 12.3^{0}C. The minimum average temperature for this period was 10.2^{0}C for 2021, and the maximum 14.6^{0}C for 2020. The average temperature of November was 5.48^{0}C. The minimum average temperature for this period was 2.1°C for 2018, and the maximum 7.9^{0}C for 2019. The average temperature of December was 1.72^{0}C. The minimum average temperature for this period was -0.8^{0}C for 2018, and the maximum 3.5°C for 2019 (Figure 1).

During the analysed period, 2017-2022, in the South and South-East part (Valul lui Traian and Stefan Regions), the average annual temperature was 12.13^{0}C. The minimum average temperature for this period was 11.4^{0}C for 2021, and the maximum 13.1^{0}C for 2020 (Figure 2).

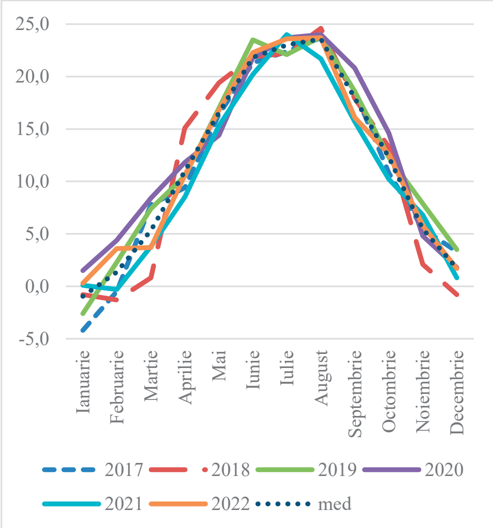

Figure 1. The mean temperature in the Central part (Codru Region) of the Republic of Moldova for the 2017-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

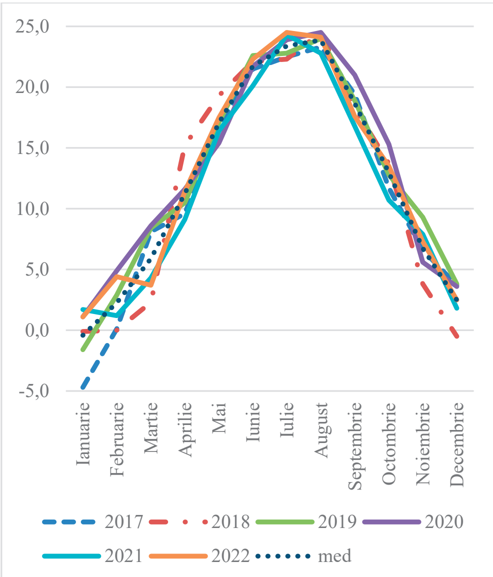

Figure 2. The mean temperature in the South and South-East part (Valul lui Traian and Stefan Regions) of the Republic of Moldova for the 2017-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

In the period, 2017-2022, the average temperature of January was -0.42^{0}C. The minimum average temperature for this period was -4.7^{0}C for 2017, and the maximum 1.7^{0}C for 2021. The average temperature of February was 2.25^{0}C. The average minimum temperature for this period was 0^{0}C for 2018, and the maximum 4.9^{0}C for 2020. The average temperature of March was 5.9^{0}C. The minimum average temperature for this period was 2.3^{0}C for 2018, and the maximum 8.6^{0}C for 2020. The average temperature of April was 11.23^{0}C. The minimum average temperature for this period was 9.1^{0}C for 2021, and the maximum 15.2^{0}C for 2018. The average temperature of May was 17.07^{0}C. The minimum average temperature for this period was 15.4^{0}C for 2020, and the maximum 19.3^{0}C for 2018. The average temperature of June was 21.72^{0}C. The minimum average temperature for this period was 20.1^{0}C for the year 2021, and the maximum 22.6^{0}C for the year 2019. The average temperature of July was 23.38^{0}C. The average minimum temperature for this period was 22.3^{0}C for the year 2019, and the maximum 24.5^{0}C for the year 2022. The average temperature of August was 23.87^{0}C. The minimum average temperature for this period was 22.8^{0}C for 2021, and the maximum 24.5^{0}C for 2020. The average temperature of September was 18.67^{0}C. The minimum average temperature for this period was 16.8°C for the year 2021, and the maximum 21.0^{0}C for the year 2020. The average temperature of October was 12.95°C. The minimum average temperature for this period was 10.7^{0}C for 2021, and the maximum 15.3°C for 2020. The average temperature of November was 6.75°C. The minimum average temperature for this period was 3.8^{0}C for 2018, and the maximum 9.3^{0}C for 2019. The average temperature of December was 2.48^{0}C. The minimum average temperature for this period was -0.5^{0}C for 2018, and the maximum 3.8^{0}C for 2019.

In the analysed period 2017-2022, in the Central part (Codru Region), the deviation of the average monthly temperature compared to the previous year of the same period (Figure 3), had values between -1.8 and 4.1^{0}C for the month of January. For the February, the average monthly temperature deviation compared to the previous year of the same period, had values between -4.7 and 3.9^{0}C, March, the values between -7 and 6.6^{0}C, in April, the values between -4.6 and 5.7^{0}C. In the May, the average monthly temperature deviation compared to the previous year of the same period, had values between -2.6 and 3^{0}C. In the June, the average monthly temperature deviation compared to the previous year of the same period, had values between -1.7 and 2.1^{0}C. In the July, the average monthly temperature deviation compared to the previous

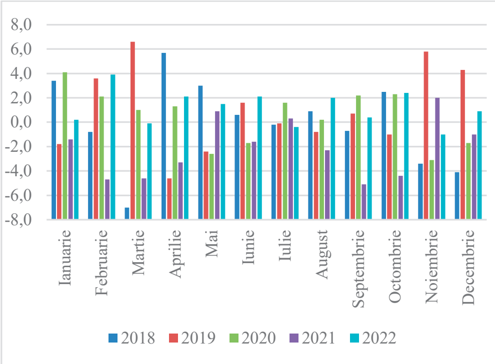

Figure 3. The difference in average temperature, for the same period, compared to the previous year in the Central part (Codru Region) of the Republic of Moldova for the 2018-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

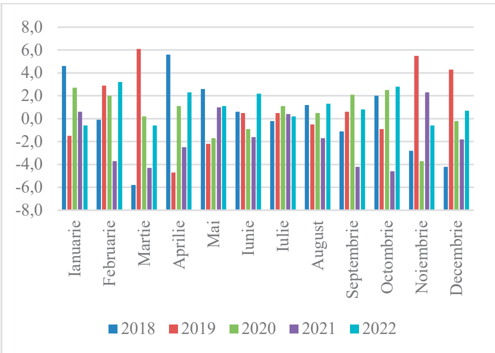

Figure 4. The difference in average temperature, for the same period, compared to the previous year in the South and South-East part (Valul lui Traian and Stefan Regions) of the Republic of Moldova for the 2018-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

year of the same period, had values between -0.4 and 1.6°C. In the August, the average monthly temperature deviation compared to the previous year of the same period, had values between -2.3 and 2^{0}C. In the September, the average monthly temperature deviation compared to the previous year of the same period, had values between -5.1 and 2.2^{0}C. For the month of October, the average monthly temperature deviation compared to the previous year of the same period, had values between -4.4 and 2.5^{0}C. For the month of November, the average monthly temperature deviation compared to the previous year of the same period, had values between -3.4 and 5.8^{0}C. In the December, the average monthly temperature deviation compared to the previous year of the same period, had values between -4.1 and 4.3^{0}C.

In the analysed period 2017-2022, in the South and South-East part (Valul lui Traian and Stefan Regions), the deviation of the average monthly temperature compared to the previous year of the same period (Figure 4), had values between -1.5 and 4.6^{0}C for the month of January. In February, the values between -3.7 and 3.2^{0}C, in March, the values between -5.8 and 6.1^{0}C, in April, the values between -4.7 and 5.6^{0}C, in May, the values between -2.2 and 2.6^{0}C, in June, the values between -1.6 and 2.2°C. For the July, the average monthly temperature deviation compared to the previous year of the same period, had values between -0.2 and 1.1^{0}C, in August, the values between -1.7 and 1.3^{0}C, in September, the values between -4.2 and 2.1^{0}C, in October, the values between -4.6 and 2.8°C, in November, the values between -3.7 and 5.5^{0}C and in December, the average monthly temperature deviation compared to the previous year of the same period, had values between -4.2 and 4.3^{0}C.

Based on the multi-year data, a trend of increasing the average annual and monthly temperature and reducing the minimum temperatures is established.

The existing global warming makes the vegetation period shorter, the winemaking campaign starts earlier in the case of wine varieties.

The reduction of absolute minimum temperatures requires technological elements to increase the resistance of plants to frost and the selection of varieties with increased resistance to frost.

For the analysed period 2017-2022, the amount of precipitation in the Central Region (Codru) constituted values between 403 and 666 mm, and for the South and South-East Region, the precipitation was within the limits of 352 and 574 mm. The average amount of precipitation for the Central Region (Codru) was 553.17 mm, and for the South and South-East - 448.33 mm (Figure 5).

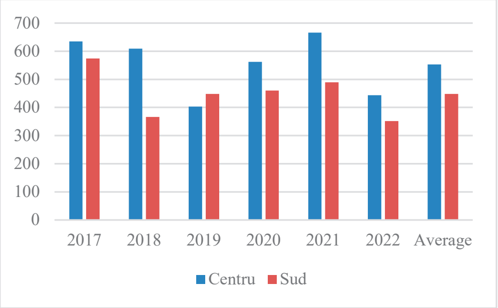

Figure 5. The yearly quantity of precipitation in the Central part (Codru Region), South and South-East part (Valul lui Traian and Stefan Regions) of the Republic of Moldova for the 2017-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

For January, the average amount of precipitation in the Central Region (Codru) was 33.17 mm, the limits of the values between 9 and 83 mm, and for the South and South-East Region, the average was 18.33 mm, the limits of the values between 5 and 31 mm (Figure 6, Figure 7). For the month of February, the average amount of precipitation in the Central Region (Codru) was 31.33 mm, the limits of the values between 6-55 mm, and for the South and South-East Region, the average was 26.5 mm, the limits of the values between 3 and 63 mm. For the month of March, the average amount of precipitation in the Center Region (Codru) was 33 mm, the limits of the values between 1-103 mm, and for the South and South-East Region, the average was 25 mm, the limits of the values between 5 and 50 mm. For the April, the average amount of precipitation in the Central Region (Codru) was 46.83 mm, the limits of the values between 4 and 127 mm, and for the South and South-East Region, the average was 41.17 mm, the limits of the values between 4 and 85 mm. For May, the average amount of precipitation in the Central Region (Codru) was 50.17 mm, the limits of the values between 18-101 mm, and for the South and South-East Region, the average was 44.33 mm, the limits of the values between 28-77 mm.

For the month of June, the average amount of precipitation in the Central Region (Codru) was 80.83 mm, the limits of values between 7 and 151 mm, and for the South and South-East Region, the average was 80.33 mm, the limits of values

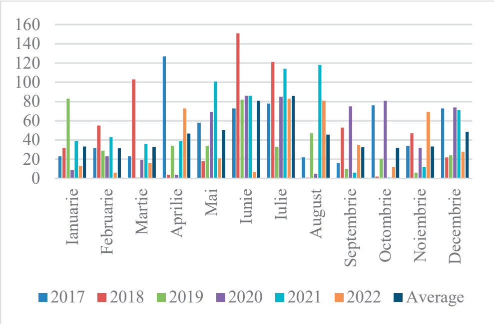

Figure 6. The month quantity of precipitation in the Central part (Codru Region) of the Republic of Moldova for the 2017-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

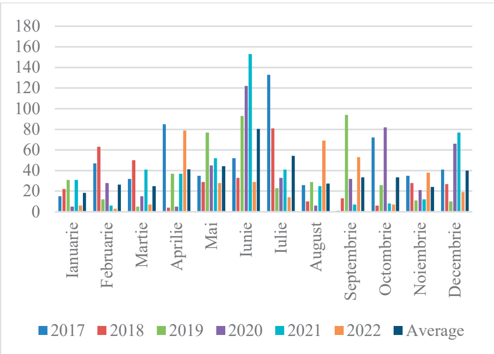

Figure 7. The month quantity of precipitation in the South and South-East part (Valul lui Traian and Stefan Regions) of the Republic of Moldova for the 2017-2022 years
Source: NBS & local meteorological stations (i-Metos), processed by authors

between 29 and 153 mm. For the month of July, the average amount of precipitation in the Central Region (Codru) was 85.67 mm, the limits of the values between 33 and 121 mm, and for the South and South-East Region, the average was 54.17 mm, the limits of the values between 14 and 133 mm. For the month of August, the average amount of precipitation in Central Region (Codru) was 45.67 mm, the limits of the values between 1 and 11 mm, and for the South and South-East Region, the average was 27.5 mm, the limits of the values between 6 and 69 mm. For the month of September, the average amount of precipitation in the Central Region (Codru) was 32.5 mm, the limits of the values between 6 and 75 mm, and for the South and South-East Region, the average was 33.33 mm, the limits of the values between 1 and 94 mm. For the month of October, the average amount of precipitation in the Central Region (Codru) was 32 mm, the limits of the values between 1 and 81 mm, and for the South and South-East Region, the average was 33.5 mm, the limits of the values between 6 to 82 mm. For the month of November, the average amount of precipitation in the Central Region (Codru) was 33.33 mm, the limits of the values between 6 to 69 mm, and for the South and South-East Region, the average was 24.17 mm, the limits of the values between 11-38 mm. For the December, the average amount of precipitation in the Central Region (Codru) was 48.67 mm, the limits of the values between 22 and 74 mm, and for the South and South-East Region, the average was 40 mm, the limits of the values between 10 and77 mm.

Based on the multi-year data, a tendency is established to reduce the annual amount of precipitation, which makes it saincreasingly necessary to irrigate the vineyards.

CONCLUSIONS

The average temperature in the analysed years was 11.6^{0}C for the Central Region (Codru) and 12.1^{0}C for the South and South-East Region.

Based on the multi-year data, a trend of increasing the average annual and monthly temperature and reducing the minimum temperatures is established.

The existing global warming makes the vegetation period shorter and the winemaking campaign starts earlier in the case of wine varieties.

The reduction of absolute minimum temperatures requires technological elements to increase the resistance of plants to frost and the selection of varieties with increased resistance to frost.

The average amount of precipitation in the analysed years was 553 mm for the Center Region (Codru) and 448 mm for the South region.

Based on the multi-year data, a tendency is established to reduce the annual amount of precipitation, which makes it increasingly necessary to irrigate the vineyards.

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EFFECT OF MIXED CULTURE WITH *TORULASPORA DELBRUECKII* AND *SACCHAROMYCES CEREVISIAE* ON PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF YOUNG WINES

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Abstract

The present study aimed to evaluate the effect of pure and mixed culture fermentations with autochtonous yeast strains Torulaspora delbrueckii and Saccharomyces cerevisiae on physico-chemical and sensory qualities of Fetească albă and Fetească neagră young wines, at pilot scale. Yeast strains were isolated during different stages of spontaneous alcoholic fermentation and selected for their potential role in winemaking: 26 strains, 17 Saccharomyces cerevisiae strains and 9 non-Saccharomyces strains, from genera Candida, Torulaspora and Debaryomyces, were evaluated in terms of extracellular enzymatic activity, fermentative and technological characteristics. From these, two Saccharomyces cerevisiae strains were selected for esterase activity, and two non-Saccharomyces strains, Torulaspora delbrueckii species were selected for ß-glucosidase and esterase activity. Different yeast strains influenced the physico-chemical characteristics of the wines. The sensory qualities of the young wines produced with mixed cultures were positively influenced.

***Key words**: mixed fermentation, wine yeasts, Torulaspora delbrueckii, Saccharomyces.*

INTRODUCTION

In the winemaking, commercial yeast strains of *Saccharomyces cerevisiae* are currently used in the form of active dry yeasts, which, if they are properly selected, ensure a fast fermentation with reduced risks.

These yeast strains have been selected from certain vine-growing areas of the world based on previously established criteria and there is currently a very wide offer of such products that are used to produce certain types of wines.

However, it was found that the wines produced with *S. cerevisiae* monocultures have shown a uniformity of the characters and a decrease in their aromatic complexity (Lambrechts et Pretorius, 2000; Rodriguez, 2010; Romano et al., 2003), while the natural fermentation of the musts leads to the production of wines of higher quality, with improved sensory properties.

Thus, in order to reproduce the conditions which characterized natural fermentations, numerous researches have been focused on the isolation and obtaining of pure culture of yeasts from non-*Saccharomyces* species.

Their screening according to oenological and technological properties highlighted the fact that not all strains from non-*Saccharomyces* species produce secondary metabolited with harmfull effects during fermentation (acetic acid, acetaldehyde, acetoin, ethyl acetate) and some of them produce and secrete several enzymes (esterases, glycosidases, lipases, b-glucosidases, proteases, cellulases, etc.) that could have a positive influence on the characteristics of the wine, mainly on the varietal aroma (Ciani et al., 2006; Gaensly et al., 2015; López et al., 2015; Strauss et al, 2001).

In recent years, new fermentation technologies based on the co-inoculation of non-*Saccharomyces* and *S. cerevisiae* yeast strains, or their sequential inoculation at different times were developed in order to improve the wines quality and complexity (Comitini et al., 2011; Contreras et al., 2014; González-Royo et al., 2015).

Torulospora delbrueckii strains have been frequently used in the last decade in the fermentation processes with sequential inoculation. Numerous studies put into evidence the ability of these strains to increase the aromatic complexity of wines (Agarbati et al., 2020;), to reduce the SO_2 content (Agarbati et al., 2020; Gonzalez-Royo et al., 2014), to increase the content in glycerol (Gonzalez-Royo et.al., 2014).

In Romania, until now, there has been a real interest and results in the isolation and oenological characterization only of yeast strains belonging to the genus *Saccharomyces*, very few works regarding the screening of non-*Saccharomyces* species, as well as the use of mixed cultures in the winemaking were reported (Brîndușe et al, 2020; Marian I. et al., .2022; Nechita, A. et al., 2020).

The present study aimed to evaluate the effect of mixed culture fermentations with autochtonous yeast strains *T. delbrueckii* and *S.cerevisiae* on physico-chemical and sensory qualities of Fetească albă and Fetească neagră young wines, at pilot scale.

MATERIALS AND METHODS

Twenty-six yeast strains belonging to *Saccharomyces*, *Candida* and *Debaryomyces* genus were screened for the production of extracellular b-glucosidase, esterase, pectinase and protease activity. From these, two *S.cerevisiae* strains, respectively 56 and 76, were selected for esterase activity, and two non-*Saccharomyces* strains, *Torulaspora rosei* species were selected for ß-glucosidase (strain 75) and esterase activity (strain 47) (Ion Marian et al., 2022). These strains were used in mixed culture fermentations using as raw material grapes from Fetească albă and Fetească neagră autochtonous varieties as fellow:

- *T. delbrueckii*, strain 75 + *S.cerevisiae*, strain 52 in case of Fetească albă variety;
- *T. delbrueckii,* strain 47 + *S. cerevisiae*, strain 76 for Fetească neagră variety.

The grapes were harvested at their physiological and technological maturity, when the must obtained from grapes of Fetească albă had a concentration in sugars of 225.6 g/L, a total acidity of 3.82 g/L tartaric acid, pH 3.9 and the must obtained from grapes of Fetească neagră showed a concentration in sugars of 235.6 g/L, a total acidity of 4.70 g/L tartaric acid pH 4.5.

Fermentations were carried out according to the following variants:

- monoculture with *S. cerevisiae* strain (10^6);
- co-inoculation of non-*Saccharomyces* strain (10^5) and a *S.cerevisiae* strain (10^6), simultaneous inoculation;
- sequential culture with inoculation of non-*Saccharomyces* strain (10^5) and a *S. cerevisiae* strain at an interval of 24 hours (10^6).

S. cerevisiae commercial strains (10^6) were inoculated as control.

Each experimental variant was represented by 10 kg of grapes. All fermentations were carried out in triplicate. The monitoring of the fermentations was carried out by daily determination of the following parameters: temperature (°C), density (g/L) and concentration in sugar of the must (g/L).

Density of the must was determined according to OIV method (OIV-MA-AS2-01B). The sugar concentration was determined by using a hand held digital refractometer and the results were expressed as an absolute value (OIV-MA-AS2-02 method). The completion of the fermentation process was established when the density value was 1000 g/L.

The young wines were analysed physico-chemically 6 months after grapes processing. The base parameters were determined according to OIV methods (Compendium of international methods of analysis, OIV 2022) are: alcoholic strength (OIV-MA-AS313-01 A), total acidity (OIV-MA-AS313-01), volatile acidity (OIV-MA-AS313-02), reducing sugar (OIV-MA-AS311-01A), total dry extract and unreducing extract (OIV-MA-AS2-03A).

The red wines were also analysed from phenolic profiles and color properties point of view.

The total phenolic content of the red wines was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The results were expressed in gallic acid equivalents, using gallic acid standard curve (0 - 0.1 mg mL^{-1}).

The anthocyanins determination was based on their transformation, under the action of bisulfite ion, into colourless derivatives (Ribéreau-Gayon and Stonestreet, 1965).

Condensed tannins were determined by the vanillin assay, with the absorbance measured at 500 nm (Swain and Hillis, 1959).

The determination of catechins (flavan 3-ols) is based on the reaction of the phloroglucinol ring with vanillin that produces a red colour with a maximum absorption at 500 nm (Pompei and Peri, 1971).

Phenolic acids were assessed according to Somers et al. (Somers, 1991) and is expressed as caffeic acid equivalents.

Chromatic parameters were determined according to the UV/Vis spectrophotometry method proposed by Glories (1984).

The sensory evaluation of wines was achieved by tasting organoleptic analysis, each sensory characteristics being appreciated by points at 1 to 5. Data processing was achieved by Dunnett t-tests, which treats one group as a control and compare all other groups against it, with a significance level of 0.05 or 0.01.

RESULTS AND DISCUSSIONS

The dynamic of the fermentation processes

For the Fetească albă variety, the fermentation process lasted for 9-14 days, with a must mass temperature of 18-21ºC (Figure 1).
The two strains of *S. cerevisiae* used as monoculture treatments, represented by *S.cerevisiae* - commercial yeast, used as a control variant, and *S.cerevisiae* - strain 52, started the fermentation process in the first 24 hours. The fermentation lasted 11 days for the control variant and 9 days in the fermentation conditions with the strain 52.

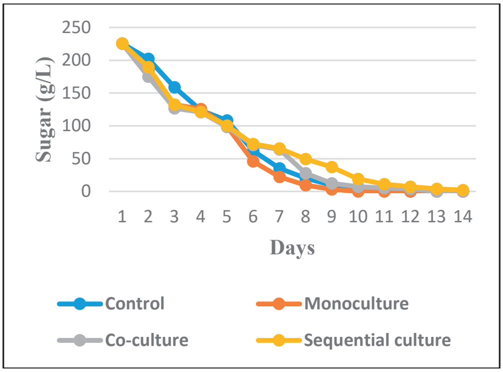

Figure 1. The sugar metabolisation curve during Fetească albă fermentation

The fermentation speed was around 20.16 g/L sugars/24 hours in the control and 24.04 g/L sugars/24 hours under fermentation conditions with strain 52. For the co-culture and sequential culture conditions, the fermentation had a similar behaviour in the first 6-7 days, after which, the fermentation speed decreased constantly until the end of the process, which lasted totally 12-14 days. Fermentation rate averaged 18.47 g/L sugars/24 h in the co-culture variant and 15.97 g/L sugars/24 h in the sequential culture variant.
Regarding the Fetească neagră variety, the fermentation was finished after around 10 - 12 days, the temperature of the must mass being around 17-21ºC (Figure 2). The process lasted 11 days in the control and 10 days in the fermentation conditions with the *S. cerevisiae* - strain 76. The fermentation rate was on average 21.05 g/L sugars/24 hours in the control variant and 23.38 g/L sugars/24 hours under fermentation conditions with strain 76. Under co-culture and sequential culture conditions, the fermentation lasted 12 days with a speed of around 19.18 g/L sugars/24 h, respectively 19.40 g/L sugars/24 h.

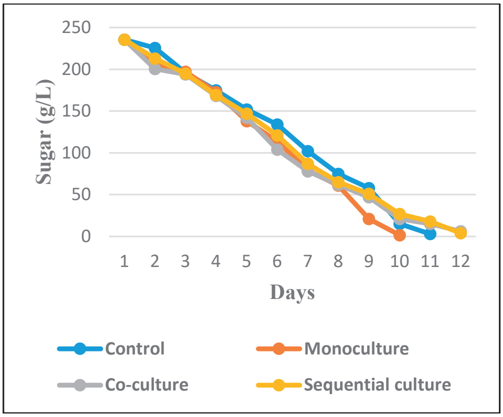

Figure 2. The sugar metabolisation curve during Fetească neagră fermentation

The physicochemical parameters of wines, depending on the different starter cultures used in fermentations are presented in Table 1.

Table 1. Physicochemical composition of wines

Grapevine variety	Parameters	Variants			
		Control	Monoculture	Co-culture	Sequential culture
Feteasca Alba	Alcohol content (% vol)	12.3±0.04	13±0.02**	12.3±0.03	12.5±0.06
	Sugar (g/L)	3.8±0.01	3.4±0.02	4±0.01**	2±0.03
	Total acidity (g/L tartaric ac.)	3.68±0.03	4.58±0.04**	5±0.02**	4.8±0.02**
	Volatile acidity (g/L CH_3COOH)	0.48±0.02	0.6±0.00	0.26±0.01**	0.58±0.02
	Total dry extract (g/L)	26.1±0.05	29.0±0.01**	24.8±0.01	24.8±0.04
	Unreduced extract (g/L)	21.29±0.03	24.9±0.01	20.8±0.00*	22.8±0.03
Feteasca Neagra	Alcohol content (% vol)	13.7±0.02	14±0.04	13.9±0.00	14±0.00
	Sugar (g/L)	3±0.03	1.62±0.02	6.06±0.04**	4±0.00**
	Total acidity (g/L tartaric ac.)	4.28±0.03	4±0.00	4.36±0.01	4.43±0.02**
	Volatile acidity (g/L CH_3COOH)	0.51±0.01	0.5±0.02	0.28±0.02**	0.46±0.04
	Total dry extract (g/L)	26.8±0.04	25.3±0.07	27.5±0.01**	26.3±0.05
	Unreduced extract (g/L)	23.8±0.01	23.7±0.03	19.44±0.01*	21.68±0.00

*The mean difference is significant at the P= 0.05 level;
**The mean difference is significant at the level P > 0.001 according to Dunnett t-tests.; [a] C.y. – commercial yeast

All fermentations with mixed inoculum of non-*Saccharomyces* and *Saccharomyces* (co-culture

and sequential culture) were completed with less than 4g/L residual sugars except Fetească neagră co-culture variant with a higher concentration of residual sugar, respectively 6.06 g/L, the wines obtained being semi dry.
The titratable acidity of white wine samples was higher in co-culture, sequential culture and monoculture variants compared with the control. For the red wine samples, all treatments produced similar titratable acidity values, which ranged between 4 g/L in monoculture variant and 4.43 g/L in the co-culture variant. The volatile acid content is very important for the wines health and is recommended to register a low value, best result being obtained after the co-culture treatment in both white and red wines.
The values of total dry extract were higher for Fetească alba wines fermented with authtonous *S. cerevisiae*, strain 52 and for Fetească neagră wines fermented in co-culture with *T.delbrueckii,* strain 47 and *S. cerevisiae*, strain 76.
The lowest values of non-reducing extract were registered for both types of wine in co-culture fermentation.
Concerning Fetească neagră wines, the achieved results indicated significant differences in the level of phenolic compounds between control and wines produced with *T.delbrueckii,* strain 47 + *S. cerevisiae*, strain 76 in different variants.
The influence of yeast strains combination on the anthocyanins profile was noted especially in case of co-culture variant, which significantly registered the highest value. In monoculture and sequential culture, values of anthocyanins closed to the control were obtained.
A higher content in phenolic acid, statistically assured compared to the control were observed in all fermentations (Table 2).
The most intense color was shown by Fetească neagră co-culture variant which registered also the highest content in anthocyanins. Lower intensity closed to the control was observed in monoculture variant.
The lowest tonality was observed in sequential culture. Similar values were recorded in the case of the other variants (Table 3).

Table 2. Polyphenolic compounds of Fetească neagră wines

Parameters	Variants			
	Control	Monoculture	Co-culture	Sequential culture
Catechins (mg/L)	0.21±0.1	0.23±0.00	0.21±0.00	0.21±0.03
Total polyphenols (mg/ GAE/L)	4603±2	4689±0**	4634±1**	4719±2**
Anthocyanins (mg/L)	345±1.73	345±3	392±2**	344±2
Tannins (g/L)	1.82±0.01	2.11±0.01**	1.9±0.03**	2.24±0**
Catechins (mg/L)	1.16±0	1.3±0.03**	1.18±0.02	1.39±0.02**
Phenolic acid (mg. caffeic acid/L)	422±1	468±0**	482±1**	484±0**

*The mean difference is significant at the P= 0.05 level.
**The mean difference is significant at the level P > 0.001 according to Dunnett t-tests.

Table 3. Chromatic properties of Fetească neagră wines

Parameters	Variants			
	Control	Monoculture	Co-culture	Sequential culture
Colour intensity	7.12±0.01	7.14±01	7.82±0.02	7.33±0.01
Hue	0.87±01	0.87±0.02	0.82±01	0.72±0.01
d 420% (% yellow pigments)	40.73±0.04	40.62±0.01	38.87±01	36.97±0.01
d 520 % (% red pigments)	46.35±0.03	46.50±0.01	47.31±0.02	51.16±02
d 620 % (% blue pigments)	12.92±0.02	12.89±01	13.81±0.01	11.87±0.04
dA%	42.12±0.02	42.47±01	44.32±0.01	52.27±0.04

Sensory analysis of wines

As expected in young wines, the fruity-flowery aroma have prevailed.
From the sensory point of view, among the Feteasca alba wines, the wine fermented with *T. delbrueckii*, strain 75 and *S. cerevisiae*, strain 52 applied in sequential culture stood out.
The wine was appreciated as being clear, straw-yellow in color, with great intensity. The flavors were classified in the normal category, in a complex mixture, the overall intensity of the flavors being rated as average. Floral (vine flowers, wildflowers), citrus (lemon, grapefruit) and honey (propolis) flavors predominate. The wine is dry, acidic, with a faint saltiness, and a little bitter. The alcoholic strength was assessed as normal. The light astringency given by a low tannic intensity makes these wines appreciated as balanced, correct, with medium aromatic persistence (Figure 3).

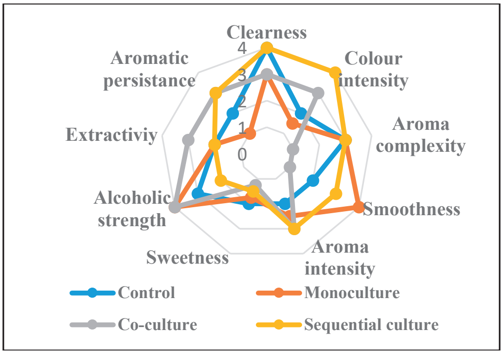

Figure 3. Sensory characteristics for Fetească albă wines

Among the Feteasca neagra wines, the wine obtained through co-culture (*T. delbrueckii*, strain 47 and *S. cerevisiae*, strain 76), stands out due to the fine flavor of black currants and pepper.

The wine was appreciated as being clear, ruby in color, with medium coloring intensity. The flavors are complex, delicate, with medium intensity. The wine is balanced, soft sweet, and with a weak salty sensation, without notes of bitterness. The alcoholic strength and extractivity were rated as normal. The wine presents a light astringency, round tannins with a normal intensity, and a correct balance. The aromatic persistence is long (Figure 4).

The enhance of aromatic intensity and complexity of Soave, Chardonnay and Vino Santo wines produced by multi-starter fermentation of *T. delbrueckii* strains and *Saccharomyces* yeasts in comparison with monoculture fermentation was also reported by Azzolini et al. (2015). Multi-starter fermentation greatly affected the content of several important volatile compounds, including 2-phenylethanol, isoamyl acetate, fatty acid esters, C_4-C_{10} fatty acids and vinylphenols which improved and enhanced wines' flavor.

The capacity of *T. delbrueckii* in sequential fermentations with *S. cerevisiae* to improve the aromatic complexity of wines by increasing their fruity flavour, while keeping spoilage attributes (volatile acidity, ethyl acetate and acetaldehyde) at suitable levels was reported by Loira et al. (2014).

According to Nechita et al. (2022) *T. delbrueckii/S. cerevisiae* yeast association at micropilot level led to a decrease in volatile acidity and an increase in glycerol and aroma compound concentrations.

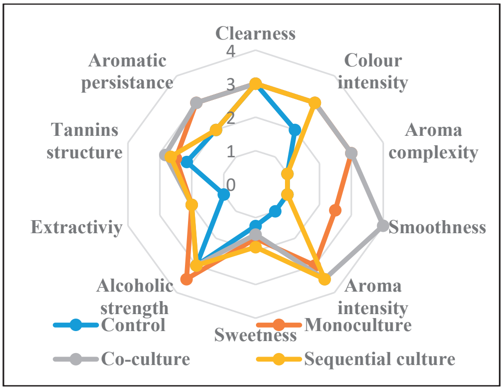

Figure 4. Sensory characteristics for Fetească neagră wines

CONCLUSIONS

The results presented in this study highlight the potential of autochthonous *Saccharomyces cerevisiae* strains in mixed culture with *Torulaspora delbrueckii* strains to enhance wines quality due to their impact on physico-chemical parameters and, especially, on the sensory properties of the wines. All fermentations with mixed inoculum of non-*Saccharomyces* and *Saccharomyces* (co-culture and sequential culture) were completed with less than 4 g/L residual sugars except Fetească neagră co-culture variant with a higher concentration of residual sugar, respectively 6.06 g/L, the wines obtained being semi dry. Significant differences between variants were recorded concerning some physico-chemical parameters and the polyphenolic compounds. Sensory analysis of wines pointed out the ability of *T. delbrueckii* in mixed culture with *S. cerevisiae* to improve the aromatic complexity of wines by increasing their fruity-flowery flavour. Further research is necessary to optimize the potential of this strains in wine industry.

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THE TABLE GRAPES AND WINE SECTOR OF THE REPUBLIC OF MOLDOVA - ACHIEVEMENTS AND PERSPECTIVES

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Abstract

The wine sector in Moldova is strategic for the national economy. The activity of the sector is coordinated by ministry, also by the district agricultural directorates at the regional level. The wine-growing sector is divided into 4 regions with PGI: `Codru`, `Ștefan Vodă`, `Valul lui Traian` `Divin`. According to the data of the NBS, the area of vineyards is continuously decreasing, in 2021 it reached 117500 ha. By category of enterprises, it is distributed - 21.62% agricultural enterprises, 38.21% farmer farms and 40.17% family farms. The total area of vineyards with wine varieties is 99600 ha or 88.77%, but with table grapes varieties constitute 17900 ha. The average harvest was 4.54 t/ha for all types of farms. The total production of grapes in farms of all categories in 2021 was - 490700 tons, the largest total harvest in the last 5 years being recorded in 2018 with 730200 tons. Analyzing the OIV data for the period 2017-2021, we mention that in the 2018 we produced the highest volume of wine - 1900000 hl, and the lowest volume was in 2020 - 920000 hl.

Key words: *Republic of Moldova, table grapes, viticulture, varieties, wine.*

INTRODUCTION

The wine sector in Moldova is strategic for the national economy, fact stated in various scientific works (Cociorva, 2022; Cociorva, 2023; Cociorva et al., 2022; Nicolaescu et al., 2022). The activity of the sector is coordinated by ministry, also by the district agricultural directorates at the regional level. The wine-growing sector is divided into 4 regions with PGI: `Codru`, `Ștefan Vodă`, `Valul lui Traian` `Divin` (Mogîldea et al., 2021).

The development of the agri-food sector, including the grape and wine sector, is influenced by a series of factors of a different nature - technological, ecological, political, legislative etc. (Godoroja et al., 2021; Midari et al., 2021; Nicolaescu et al., 2022; Nicolaescu et al., 2023; Voinesco et al., 2023).

A particular importance in the development of the wine sector belongs to the grape varieties assortment. In Moldova, local and introduced varieties are approved, which are included in the Catalog of plant varieties (Dosca et al., 2023; Mațcu et al., 2023; Procopenco et al., 2023; Voinesco et al., 2023).

The purpose of the research reflected in this article consists in:

- studying the current state and prospects for the development of viticulture (varieties, technology, vineyards areas, yield, quality etc.);
- studying the current state and prospects for winemaking development (product range, sales market, sales incomes etc.).

MATERIALS AND METHODS

For the study of the current situation in the wine sector, the following databases and information were used:

- National Bureau of Statistics (NBS) of the Republic of Moldova;
- Ministry of Agriculture and Food Industry of the Republic of Moldova;
- National Office of Vine and Wine (NOVW);
- FAO - Faostat;
- UN - Comtrade;
- OIV;
- Official documents and special literature etc.

MS Office Excel (2019) was used for the mathematical processing of the data.

RESULTS AND DISCUSSIONS

The wine-growing sector is divided into 4 regions with PGI: `Codru`, `Ștefan Vodă`, `Valul lui Traian` `Divin` (Figure 1).

The wine-growing regions are specialized in the production of different types of wines, depending on the tradition, the pedoclimatic conditions and the skill of the winemakers.

The production of grapes and wines is coordinated by the Ministry of Agriculture and Food Industry of the Republic of Moldova, the National Office of Vine and Wine, and the quality is monitored by the National Agency for Food Safety.

Figure 1. The wine-growing regions of Moldova
Source: https://ecopresa.md/wp-content/uploads/2019/07/harta-zone-vinicole-Moldova.jpg processed by authors

During the years 2000-2022, the minimum vineyards area was 114.12 thousand hectares in 2020, and the maximum vineyards area was 149.69 thousand hectares in 2001 (Figure 2, Table 1).

In the 2022, the vineyards area constituted 130 thousand hectares, or by approx. 7.3 thousand hectares more than in the 2021.

In the year 2022, from the world vineyards areas (6729.2 thousand hectares), the vineyards areas of the Republic of Moldova constitute approx. 1.82%, and from the Europe vineyards areas (3432.12 thousand hectares), the vineyards areas of the Republic of Moldova constitute approx. 3.56%.

The development trend of the vineyard areas is decreasing, according to the trend line (Figure 2).

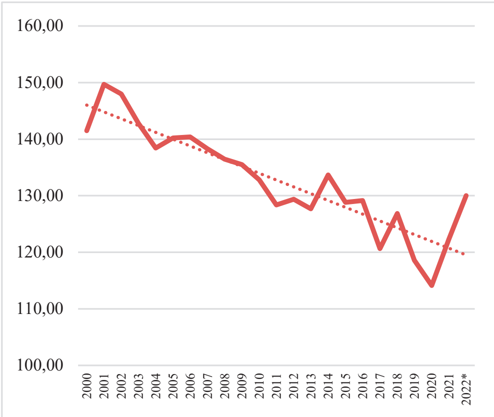

Figure 2. The vineyards area (thousand hectares) and trendline in the Republic of Moldova during the 2000-2022 years
Source: NBS & FAOSTAT, processed by authors

Table 1. Vineyards areas, yield and grape production in the Republic of Moldova during the 2000-2022 years

Year	Vineyards areas		Yield		Grape production	
	1000 ha	% compared to the previous year	t/ha	% compared to the previous year	1000 t	% compared to the previous year
2000	141.49	100.00	4.97	100.00	703.79	100.00
2001	149.69	105.80	3.37	67.81	505.01	71.76
2002	148.00	98.87	4.33	128.49	641.16	126.96
2003	142.80	96.49	4.74	109.47	677.20	105.62
2004	138.44	96.95	4.95	104.43	685.57	101.24
2005	140.21	101.28	3.70	74.75	518.53	75.63
2006	140.39	100.13	3.32	89.73	466.06	89.88
2007	138.27	98.49	4.32	130.12	597.96	128.30
2008	136.47	98.70	4.66	107.87	635.51	106.28
2009	135.50	99.29	5.06	108.58	685.10	107.80
2010	132.81	98.01	3.63	71.74	481.62	70.30
2011	128.35	96.64	4.63	127.55	594.84	123.51
2012	129.35	100.78	3.91	84.45	505.92	85.05
2013	127.68	98.71	4.80	122.76	612.70	121.11
2014	133.67	104.69	4.44	92.50	593.88	96.93
2015	128.83	96.38	4.65	104.73	598.66	100.80
2016	129.13	100.23	4.77	102.58	615.74	102.85
2017	120.66	93.44	5.59	117.19	675.06	109.63
2018	126.87	105.15	5.76	103.04	730.17	108.16
2019	118.59	93.47	5.55	96.35	658.73	90.22
2020	114.12	96.23	4.05	72.97	462.05	70.14
2021	122.28	107.15	4.39	108.40	536.83	116.18
2022	130.00	106.31	5.50	125.28	715.00	133.19

Source: NBS & FAOSTAT & OIV, processed by authors

During the years 2000-2022, the minimum grapes' production was 462.05 thousand tonnes in 2020, and the maximum grapes' production was 730.17 thousand tonnes in 2018 (Figure 3, Table 1).

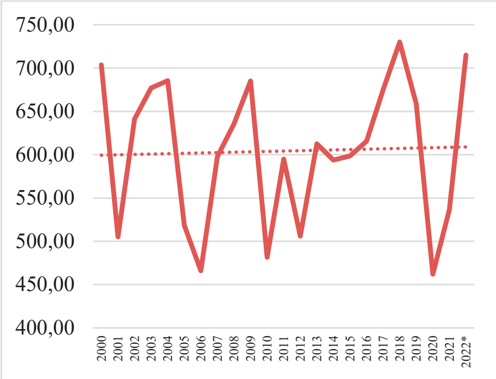

Figure 3. The grapes production (thousand tons) and trendline in the Republic of Moldova during the 2000-2022 years
Source: NBS & FAOSTAT, processed by authors

In 2022, grapes' production was 715 thousand tons, or approx. 178.17 thousand tons more than in 2021.
In the world grapes' production (73524.2 thousand tons), the grapes' production of the Republic of Moldova is approx. 0.73%, and from the Europe grapes' production (26926.34 thousand tons), the grapes' production of Moldova has approx. 1.99%.
The development trend of grape production is at the level of 600 thousand tons with a slight increase, according to the trend line.
In the period 2000-2022, the minimum grapes yield was 3.32 t/ha in 2006, and the maximum grapes yield was 5.76 t/ha in 2018 (Figure 4, Table 1).
In 2022, the grapes yield was 5.5 t/ha, or by approx. 1.1 t/ha more than in 2021.
The average grapes yield in the world was 10.93 t/ha in 2021, the average grapes yield in the Europe was 7.85 t/ha, and in Moldova - 4.39 t/ha.
During the years 2000-2022, the minimum wine production was 840 thousand hectolitres in 2010, and the maximum wine production was 3215 thousand hectolitres in 2003 (Figure 5, Table 2).

In 2022, wine production was 1324 thousand hectolitres, or by approx. 106 thousand hectolitres less than in 2021.

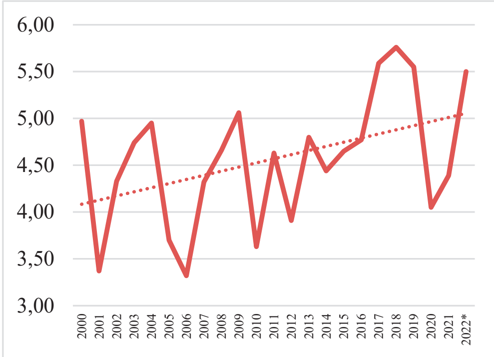

Figure 4. The grapes yield (tons/hectares) and trendline in the Republic of Moldova during the 2000-2022 years
Source: NBS & FAOSTAT, processed by authors

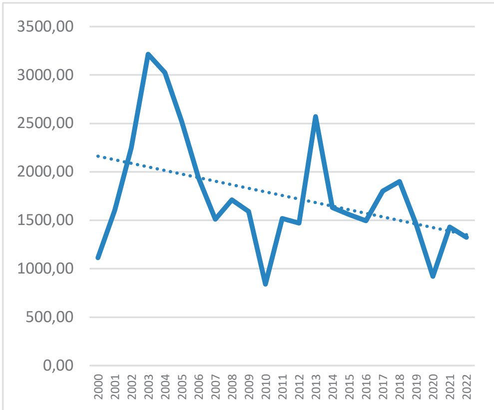

Figure 5. The wine production (thousand hectolitres) and trendline in the Republic of Moldova during the 2000-2022 years
Source: NBS & OIV, processed by authors

Table 2. Production and export of wine in the Republic of Moldova during the 2000-2022 years

Year	Production of wine				Export of wine	
	1000 t	% compared to the previous year	1000 hl	% compared to the previous year	1000 hl	% compared to the previous year
2000	111.20	100.00	1112.0	100.00	992.0	100.00
2001	160.40	144.24	1604.0	144.24	1368.0	137.90
2002	154.20	96.13	2251.0	140.34	1537.0	112.35
2003	197.90	128.34	3215.0	142.83	2022.0	131.55
2004	340.20	171.91	3026.0	94.12	2280.0	112.76
2005	373.40	109.76	2520.0	83.28	2425.0	106.36
2006	193.81	51.90	1938.0	76.90	1460.0	60.21
2007	128.49	66.30	1510.0	77.92	660.0	45.21

Year	Production of wine				Export of wine	
	1000 t	% compared to the previous year	1000 hl	% compared to the previous year	1000 hl	% compared to the previous year
2008	159.70	124.29	1710.0	113.25	899.0	136.21
2009	130.00	81.40	1590.0	92.98	957.0	106.45
2010	132.56	101.97	840.0	52.83	1260.0	131.66
2011	131.86	99.47	1520.0	180.95	1198.0	95.08
2012	147.54	111.89	1470.0	96.71	1220.0	101.84
2013	158.96	107.74	2570.0	174.83	1234.0	101.15
2014	149.85	94.27	1630.0	63.42	1171.0	94.89
2015	139.51	93.10	1560.0	95.71	1220.0	104.18
2016	141.46	101.40	1495.0	95.83	1328.0	108.85
2017	171.43	121.19	1801.0	120.47	1405.0	105.80
2018	178.49	104.12	1900.0	105.50	1399.0	99.57
2019	182.70	102.36	1460.0	76.84	1567.0	112.01
2020	178.70	97.81	920.0	63.01	1330.0	84.88
2021	-	-	1430.0	155.43	1207.0	90.75
2022	-	-	1324.0	92.59	1041.0	86.25

" - " no data

Source: NBS & FAOSTAT & OIV, processed by authors

The development trend of wine production is decreasing, according to the trend line (Figure 5).

During the years 2000-2022, the minimum exported wine production was 660 thousand hectolitres in 2007, and the maximum exported wine production was 2425 thousand hectolitres in 2005 (Figure 6, Table 2).

In 2022, exported wine production was 1041 thousand hectolitres, or by approx. 166 thousand hectolitres less than in 2021.

The development trend of wine production export is decreasing, according to the trend line.

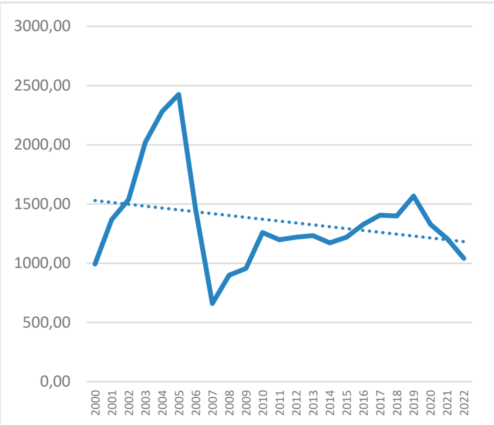

Figure 6. The export of wine (thousand hectolitres) and trendline in the Republic of Moldova during the 2000-2022 years
Source: NBS & OIV, processed by authors

Table 3. Trade balance, export vs import (1000 USD) of grapes and wine production in the Republic of Moldova during the 2000-2021 years

Year	Grapes			Wine		
	Import Value	Export Value	Trade balance	Import Value	Export Value	Trade balance
2000	0.00	1.61	1.60	0.92	87.95	87.04
2001	0.01	1.55	1.54	1.69	124.37	122.68
2002	0.12	1.59	1.47	7.64	136.64	129.00
2003	0.16	3.41	3.25	5.81	180.88	175.06
2004	0.21	2.46	2.25	5.53	215.85	210.33
2005	1.71	2.45	0.75	9.71	245.00	235.29
2006	0.17	3.37	3.20	13.08	136.98	123.90
2007	0.37	12.02	11.65	2.06	83.61	81.55
2008	0.37	7.52	7.15	1.28	133.68	132.40
2009	1.44	13.57	12.13	0.93	128.19	127.26
2010	1.66	12.98	11.32	3.16	137.86	134.70
2011	5.03	17.55	12.52	2.44	131.62	129.19
2012	2.20	14.50	12.29	1.39	142.13	140.74
2013	3.28	17.02	13.74	1.94	149.57	147.63
2014	1.71	20.12	18.42	1.55	111.60	110.05
2015	1.47	16.78	15.31	1.58	97.28	95.70
2016	0.82	20.40	19.59	1.76	107.54	105.77
2017	0.36	38.63	38.27	1.62	127.67	126.05
2018	0.49	25.12	24.63	2.89	137.91	135.02
2019	0.43	29.66	29.23	2.62	139.59	136.97
2020	0.56	26.87	26.31	2.99	134.38	131.38
2021	1.03	36.08	35.05	6.04	141.24	135.20

Source: NBS & FAOSTAT, processed by authors

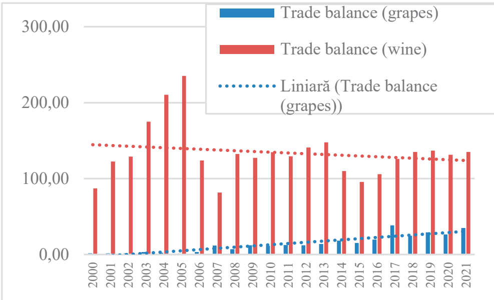

Figure 7. Trade balance, export vs import (1000 USD) of grapes and wine production, and trendline in the Republic of Moldova during the 2000-2021 years
Source: NBS & OIV, processed by authors

Analyzing the data with reference to international trade of grapes and wine, we note that a negative trend of the trade balance for wine production is observed, and a positive trend of the trade balance for grapes production (Figure 7, Table 3).

For the further development of the grape and winemaking sector, the Government and Ministry focus is on the local selection of vine varieties.

Among the varieties for table grapes, we mention the following local varieties: Moldova, Codreanca et al.; introductory varieties: Cardinal, Lora, Arkadia, etc.

Among the varieties for wine, the focus is on the following local varieties: Feteasca neagra, Feteasca alba, Feteasca regala, Rara neagra etc.; the introductory varieties: Cabernet Sauvignon, Merlot, Malbec, Pinot noir, Pinot grey, Chardonnay, Sauvignon etc.

CONCLUSIONS

The development trend of the vineyard areas is decreasing, according to the trend line.
The development trend of grape production is at the level of 600 thousand tonnes with a slight increase, according to the trend line.
The development trend of wine production is decreasing, according to the trend line.
The development trend of wine production export is decreasing, according to the trend line
For the further development of the grape and winemaking sector, the Government and Ministry focus is on the local selection of vine varieties.

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ASSESSMENT OF SOMACLONAL VARIATION IN MICROPROPAGATED GRAPEVINE CULTIVARS USING MOLECULAR MARKERS

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Abstract

Micropropagation of five grapevine cultivars (Fetească albă, Cabernet Sauvignon, Merlot, Riesling Italian and Traminer roz) was performed using axillary shoots as a source of explant from field-grown plants. Shoot initiation and proliferation were performed on Murashige and Skoog (MS) medium with 0.5 mg/L NAA and BAP (0.5, 1.0 and 2.5 BAP mg/L). Subsequently, proliferated shoots from V3 were used for grapevine callus induction. The callus was then subcultured on fresh MS medium supplemented with 1.0 mg/L BAP +5.0 (V4) and 10 mg/l NAA (V5). Embryogenic calli grown on V5 were transferred and subcultured on MS medium supplemented with 0.5 mg/L NAA and three different concentrations of TDZ (0.5; 1.0; 2.0 mg/L) for shoot regeneration. RAPD assay was performed after the 12th subculture using in vitro-raised plants from V6 culture medium (0.5 mg/L NAA+0.5 mg/L TDZ) and the mother plants grown in the field and used as control. The results of this study reveal that the highest number of somaclones (5) was regenerated from the Merlot variety and the lowest number (1) from the Feteasca albă and Traminer roz varieties. For practice, the induction of somaclonal variation and the selection of valuable somaclones are important goals for future grapevine breeding programs.

***Key words**: Vitis vinifera*, *in vitro*, callus, somaclones, RAPD.

INTRODUCTION

Grapevine (*Vitis vinifera* subsp. *vinifera*) is one of the oldest and the most important fruit crops in the world (Butiuc-Keul and Coste, 2023). There are currently over 15000 named grapevine cultivars held in *Vitis* collections worldwide (Panara et al., 2018). However, the identification and characterization of grapevine cultivars have been one of the points of interest in viticulture (Jackson et al., 2014). This objective has usually been achieved by evaluating the agrobiological and ampelographic characteristics of grapevine varieties (Stavrakaki and Biniari, 2017; Maistrenko et al., 2020). Thus, distinguishing clones of the same variety or closely related varieties by the previously mentioned characteristics was sometimes very difficult and required the existence of qualified personnel in the field of viticulture (Biniari and Stavrakaki, 2019). To complement the morphological characterization of grapevine varieties, biotechnological approaches, such as DNA-based molecular markers techniques have been developed to analyse the diversity of grape germplasm resources (Milišić et al., 2021). In this regard, several molecular markers have been used for characterization of grapevine cultivars, clone's identification and the detection of synonymies, including random amplified polymorphic DNA (RAPDs) (Karataş & Ağaoğlu, 2010), amplified fragment length polymorphisms (AFLPs) (Stajner et al., 2009), simple sequence repeats (SSRs) and single nucleotide polymorphism (SNPs) (Emanuelli et al., 2013; Villano et al., 2014).

In addition, biotechnological researches were used to improve the quality of grape cultivars (Butiuc-Keul and Coste, 2023). In this regard, the development of *in vitro* plant regeneration methods has played a crucial role in the conservation and propagation of valuable genotypes, as well as in increasing genetic variability (Butiuc-Keul et al., 2008; Fehér, 2019; Nuzzo and Perrone, 2022). To date, *in vitro* grapevine regeneration has been achieved by organogenesis and somatic embryogenesis (Fehér, 2019; Campos et al., 2021).

Organogenesis is defined as the development of organs, such as roots, shoots, and flowers, either directly from an explant (direct organogenesis), or from the callus culture (indirect organogenesis). It is noteworthy that indirect organogenesis induces somaclonal variation involving both genetic and epigenetic changes in *in vitro* regenerated plants that could be useful for the selection of improved grapevine genotypes (Schellenbaum et al., 2008). Moreover, it has been shown that PCR-based molecular markers such as RAPD have been effective for detection of occurred grapevine somaclonal variations (Pop, 2008). Thus, the aim of the present research was to study the influences of different experimental factors such as growth regulators, type of *in vitro* culture, length of culture, and genotype on somaclones obtained from five grapevine cultivars. A comparative RAPD analysis was performed between callus-regenerated somaclones from five grapevine cultivars and their field-grown mother plants.

MATERIALS AND METHODS

Plant material and *in vitro* culture

Micropropagation of five grapevine cultivars: Cabernet sauvignon, Fetească albă, Merlot, Riesling Italian and Traminer roz (Gewürztraminer) was performed using as explant sources nodal segments from field-grown plants.

Shoot initiation and proliferation were performed on Murashige and Skoog (MS) medium supplemented with 0.5 mg/L NAA + 0.5 mg/L BAP (V1); 0.5 mg/L NAA + 1.0 mg/L BAP (V2) and 0.5 mg/L NAA + 2.5 mg/L BAP (V3). The proliferated shoots on V3 were used for the induction of the grapevine callus. Calli were then subcultured on fresh MS medium supplemented with 1.0 mg/L BAP +5.0 mg/l NAA (V4) and 1.0 mg/L BAP +10 mg/l NAA (V5). The morphogenic calli grown on V5 were transferred and sub-cultured on MS medium supplemented with 0.5 mg/L NAA and three different concentrations of TDZ (0.5 mg/L -V6; 1.0 mg/L - V7; 2.0 mg/L- V8) for shoot regeneration. The *in vitro* plants regenerated via callus after the 12^{th} subculture (8 weeks/subculture) were acclimatized under greenhouse conditions in plastic pots (Ø = 9 cm) filled with perlite. The rooted plants were then hardened under field conditions.

Genetic analysis using RAPD markers

RAPD analysis was performed after the 12th subculture using the *in vitro*-raised plants from V6 culture medium (0.5 mg/L NAA+0.5 mg/L TDZ). Field-grown plants of each cultivar analysed were used as controls.

Before DNA isolation, harvested leaves from each cultivar were dried, ground into a fine powder (TissueLyser II, Qiagen, Germany), and stored at 4°C until genetic analyses were performed.

Total genomic DNA was isolated from 0.15 g of dry powder using a protocol based on the CTAB (cetyltrime-thylammonium bromide) method published by Lodhi et al. (1994) and improved by Pop et al. (2003) and Bodea et al. (2016). DNA concentration (ng/ µL) and purity (260/280 nm) were determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Before performing PCR (polymerase chain reaction) amplifications, all DNA samples were diluted to 50 ng/µL, using sterile double-distilled water. For the RAPD analysis, the PCR amplification reactions were performed using the protocol described by Williams et al. (1990) modified by Pop et al. (2003). To amplify the DNA samples, a number of 9 RAPD primers (Microsynth AG) were used. PCR amplifications were performed in 25 µl reaction volume containing 50 ng of template, 200 µM dNTP, 0.2 µM primer, 2.5 mM $MgCl_2$, 2.5 mM 10 x buffer, 1 U Taq DNA Polymerase (Promega, USA) and 2% PVP in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett Research). PCR conditions were: 1 cycle of 95ºC for 3 minutes for initial denaturation, followed by 45 cycles of 1 minute at 93ºC for denaturation, primer annealing at 34ºC for 1 minute, and primer extension at 72ºC for 1 minute. After a final extension cycle (10 min. at 72ºC) the samples were stored at 4ºC. The PCR amplifications were repeated twice for each RAPD primer to ensure the reproducibility of the results. Separation of the PCR amplified products was carried out by electrophoresis on 1.4% agarose gels (Promega, Madison, WA, USA) stained with ethidium bromide solution in 1X TBE (Tris Borate-EDTA buffer), at 110 V and 136 mA for 2.5-3 h. The electrophoretic profiles were visualized under UV (ultraviolet) in UVP Biospectrum AC Imaging System (Upland, CA, USA). The list of RAPD primers used in this study is shown in Table 1.

Table 1. The list of RAPD primers used

Primer name	The 3'-5'nucleotide sequence of the primer
OPA 01	CAG GCC CTT C
OPA 03	AGT CAG CCA C
OPA 04	AAT CGG GCT G
OPAB 11	GTG CGC AAT G
OPAB 18	CTG GCG TGT C
AB 11	GTG CGC AAT G
OPAL 20	AGG AGT CGG A
OPX 03	TGG CGC AGT C
OPE 14	TGC GGC TGA G

Statistical analysis

The data were analysed using ANOVA PoliFact software (UASVM Cluj-Napoca, 2015) with Duncan test for multiple comparisons among the experimental variants (three repetitions x 5 vessels x 5 inoculum/ variant) (p-value < 0.05). Gel images were analyzed using TL120 software (Nonlinear Dynamics). Amplified bands were scored present (1) or absent (0) and data entered into a binary matrix. The genetic distances between analysed genotypes were calculated using Euclidean coefficient of similarity. Cluster analysis was conducted with an UPGMA algorithm using PAST software (PAleontological STatistics Version 4.11, Natural History Museum, Norway). Its consistency was assessed using bootstrap method in 10000 repetitions.

RESULTS AND DISCUSSIONS

Multiplication by axillary shoots

The results of this study revealed that MS media supplemented with 0.5 mg/L 1-naphthaleneacetic acid (NAA) and different combinations of benzyl-adenine (BA) (0.5 mg/L; 1.0 mg/L and 2.5 mg/L) have influenced the average number of proliferated shoots/explant in the analysed grapevine genotypes. Thus, the highest number of proliferated shoots/explant was recorded for Cabernet Sauvignon (7.7) multiplied on the V3 proliferation medium variant (Fig.1). Our results were in agreement with those reported by Laslo et al. (2010) who stated that Cabernet sauvignon cv. showed a high *in vitro* regeneration capacity only on media supplemented with a high concentration of BA (5 mg/L). The lowest number of proliferated shoots/explant (1.18) was recorded in Fetească albă proliferated on the V1 culture medium variant. Our results are consistent with those reported by Butiuc-Keul et al. (2007) in a previous study concluding that Fetească albă cv. was the most recalcitrant cultivar to the *in vitro* multiplication conditions.

It is noteworthy that MS media supplemented with the highest concentration of BA (2.5 mg/L) generated in this study the highest number of proliferated shoots/explants for all five analysed grapevine genotypes as shown in Figure 1.

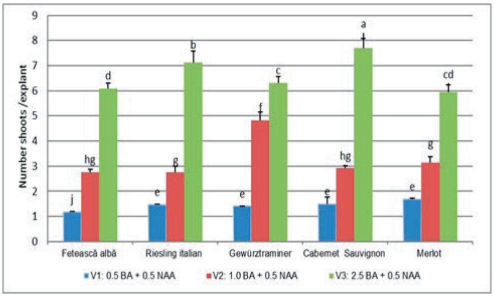

Figure 1. The influence of proliferation media variants (V1-V3) on the average number of proliferated shoots/explant in five grapevine genotypes. Bars with the same letters are not significantly different according to Duncan's multiple range test, p = 0.05. Error bars represent standard error of the mean (SEM)

However, the highest values of average length (cm) of proliferated shoots/explant were recorded on V1 culture media supplemented with the lowest concentration of BA (0.5 mg/L) (Figure 2).

Regarding the average length (cm) of proliferated shoots/explant (Figure 2), it can be seen that the longest shoots were obtained at genotype Gewürztraminer (8.22 cm), followed by Cabernet sauvignon cv. (7.14 cm) and Riesling italian cv. (6.89 cm) on the V1-variant of the proliferation culture media, while the shortest proliferated shoots were recorded in

Fetească alba (4.52 cm) on the V3-variant of the proliferation culture media (Figure 2).

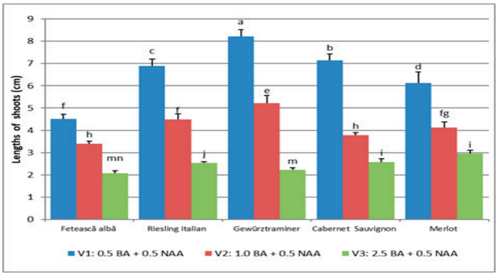

Figure 2. The influence of proliferation media variants (V1-V3) on the average length (cm) of proliferated shoots/explant in five grapevine genotypes. Bars with the same letters are not significantly different according to Duncan's multiple range test, p = 0.05. Error bars represent standard error of the mean (SEM)

An explanation of these results is due to the particularities of *in vitro* vegetative development of the analysed genotypes. According to Reisch (1986) and Garcia et al., 2023) *in vitro* proliferation of grapevine is genotype-dependent.

Callus induction

In terms of callus morphology, the results of this study revealed that explants from nodal fragments cultured on MS - variant V4 generated non-friable, white or pale-brown calli in all analysed varieties that were unusable for further experiments (Figures 3 and 4).

Figure 3. Non-morphogenic callus from Cabernet Sauvignon cv. grown on MS-V4

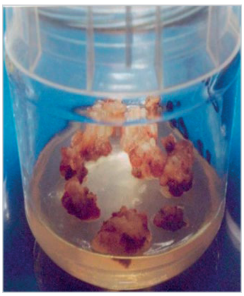

Figure 4. Non-morphogenic callus from Fetească albă cv. grown on MS-V4

Noticeably, the callus mass initiated from nodal segments and grown on V5 culture medium (10 mg/l NAA concentration) showed morphogenic characteristics (compact consistency, high optical density and different colors such as milky-white, yellow, yellow-green to red-violet pigmentation) as can be seen in Figures 5a and 5b. The amount of morphogenic callus from V5 was used for previous indirect organogenesis experiments.

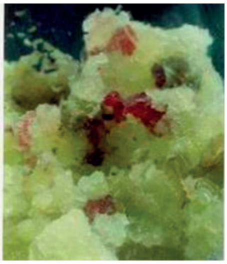

Figure 5a. Morphogenic callus from Cabernet sauvignon cv. grown on MS-V5

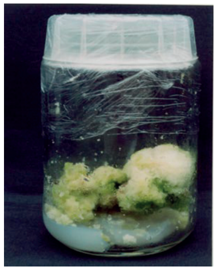

Figure 5b. Morphogenic callus from Fetească albă cv. grown on MS-V5

The results of this study showed that callus induction was successfully achieved by using the most commonly used auxins such as NAA and BA (cytokinin) for *in vitro* tissue cultures (Pop, 2008).

The influence of culture media variants (V4-V5) on the average percent (%) of induced calli in five grapevine genotypes are presented in Figure 5.

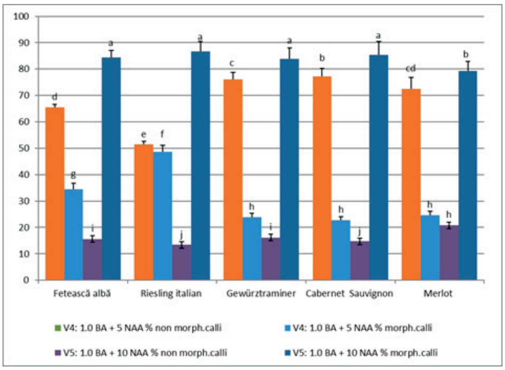

Figure 5. The influence of culture media variants (V4-V5) on the average percent (%) of induced calli in five grapevine genotypes. Bars with the same letters are not significantly different according to Duncan's multiple range test, p = 0.05. Error bars represent standard error of the mean (SEM)

In this study, morphogenic calli were induced in all five grapevine cultivars analysed on the MS-V5 culture medium variant, but with statistically non-significant differences between grapevine cultivars in terms of the percentage of morphogenic calli, except Merlot cv., as previously shown in Figure 5. These results suggest that callus induction was influenced by genotype and the combinations between the phytohormones used. In agreement with the results of this study, Yun-Zhu et al. (1985) also reported callus development in *in vitro* cultured grapevine cultivars by using different concentrations of BA and 2,4-D.

In the experiments of this study, we studied whether the plants regenerated via callus and subcultured for a long period of time were suitable for morphological and genetic changes. As shown in Figure 6, the highest average number of plantlets regenerated from morphogenic calli was recorded on MS media supplemented with 0.5 mg/L NAA + 0.5 mg/L TDZ (V6) at Riesling Italian cv. (11.6 plantlets) and Cabernet sauvignon (10.50 plantlets). The lowest average numbers were recorded at Riesling Italian cv. (3.80 plantlets) and Merlot cv. (3.97 plantlets) cultured on MS media and supplemented with 0.5 mg/L NAA + 2.0 mg/L TDZ (V8).

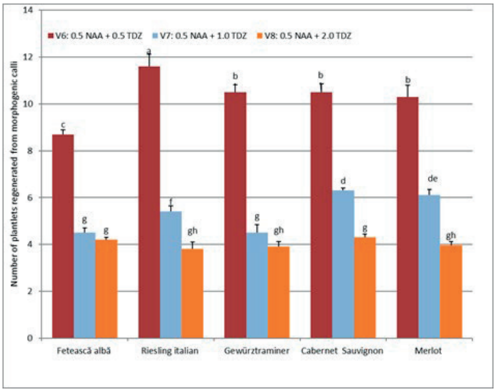

Figure 6. The influence of culture media variants (V6-V8) on the average number of plantlets regenerated from morphogenic calli in five grapevine genotypes. Bars with the same letters are not significantly different according to Duncan's multiple range test, p = 0.05. Error bars represent standard error of the mean (SEM)

These results confirm the statement that the response of grapevine to *in vitro* culture is usually related to genotype, culture medium, and plant endogenous phytohormones (Nookaraju et al., 2008; Melyan et al., 2015). Our results are consistent with other studies (Olah et al., 2003; Morariu et al., 2011) which concluded that the addition of TDZ to the culture media in concentrations ranging from 0.5-3 mg/L had a stimulatory effect on the regeneration of plants from calli, especially on woody species and recalcitrant plants.

Genetic analysis based on RAPD molecular markers

The results of the present study show that RAPD markers were suitable to assess the somaclonal variations on five grapevine cultivars. Out of the nine primers screened for their ability to amplify the DNA samples from *Vitis vinifera* subsp. *vinifera* genotypes, all revealed reproducible and consistent results. The levels of polymorphism detected with RAPD primers are presented in Table 2.

Table 2. The level of polymorphism detected with RAPD primers in somaclones from five grapevine genotypes (NPB-number of polymorphic bands; NTB-number of total bands; PPB-percent of polymorphic bands)

Primer name	Size of bands (bp)	NPB	NTB	PPB (%)
OPA 01	400-800	2	4	50
OPA 03	500-1600	4	6	66.6
OPA 04	600-1800	5	7	71.4
OPAB 11	500-2000	7	8	87.5
OPAB 18	500-1800	2	4	50.0
AB 11	600-1200	9	9	100.0
OPAL 20	500-2000	7	8	87.5
OPX 03	300-2000	7	9	77.7
OPE 14	500-1000	4	5	80.0
Total		47	60	
Average		5.2	6.6	74.5

The nine RAPD primers amplified 60 reproducible fragments ranging from 500 to 2000 bp, out of which 47 bands were polymorphic bands (5.2 bands/ primer). The number of polymorphic bands for each primer ranged from 2 to 9. The highest number of polymorphic bands (9) was generated by AB 11. The lowest number of amplified polymorphic bands (2) was obtained with the primers OPA 01 and OPAB 18. The electrophoretic profile generated with primer OPA 03 is show in Figure 7.

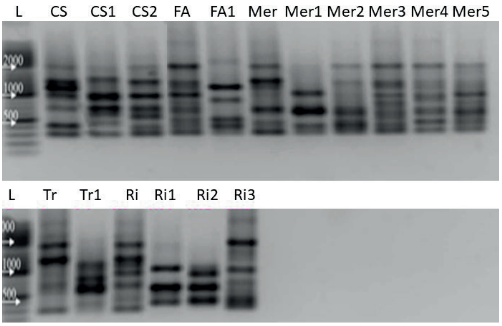

Figure 7. The genetic RAPD profiles of somaclones obtained with primer OPA 03 and generated from five grapevine genotypes. L-100 bp Ladder (Promega); CS -mother plant Cabernet Sauvignon; CS1-CS2 - somaclones from Cabernet Sauvignon; FA - mother plant Fetească albă; FA1 - somaclone Feteasca albă; Mer - mother plant Merlot; Mer1-Mer5 - somaclones from Merlot; Tr - mother plant from Traminer roz; Tr1 - somaclone from Traminer roz; Ri - mother plant from Riesling Italian; Ri1-Ri3 - somaclones from Riesling Italian

The percentage of polymorphism (no. of polymorphic bands/no. of total bands x 100) ranged from 50.0% (OPA 01 and OPAB 18) to 100.0% (AB 11) with a mean value of 74.5%.
The UPGMA dendrogram, built based on Euclidean distances, grouped the grapevine genotypes into two main clusters as shown in Figure 8. The first main cluster included one distingly sub-clusters including two somaclones (CS1 and CS2) from Cabernet Sauvignon cv. and their mother plant (CS). The second main cluster grouped five somaclones from Merlot (Mer1-Mer5) and their mother plant (Mer), one somaclone from Gewürztraminer (Tr1) and mother plant (Tr) and one somaclone from Fetească alba (FA 1) and mother plant (FA). Noteworthy is the grouping of the Italian Riesling somaclones together with their mother plant in a distinct sub-cluster compared to the other analyzed genotypes of the second main group.
This clustering pattern suggests that somaclonal variability was detected at the DNA molecular level by using RAPD molecular markers.

CONCLUSIONS

The results of this study confirm the hypothesis that after successive subcultures somaclonal variations were detected. On the other hand, micropropagation of the analyzed grapevine cultivars produced healthy and vigorous plants, which allowed them to acclimatize to *ex vitro* conditions. The callus that was induced in this study represents a valuable plant material to produce clones and make genetic improvements in *Vitis vinifera* subsp. *vinifera*.

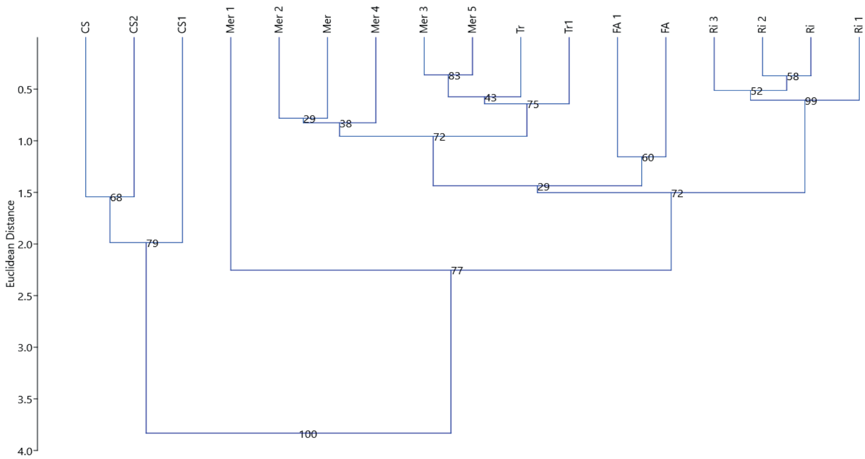

Figure 8. UPGMA dendrogram generated by RAPD markers, showing the relationships between grapevine somaclones and their mother plants based on Euclidean's distance index. Numbers on the branches show bootstrap values, computed from 10000 replications

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DETERMINATION OF THE AROMATIC PROFILE IN VARIETAL WINES FROM GRAPE VARIETIES (*VITIS VINIFERA*) GROWN IN THE DRĂGĂȘANI VINEYARD

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Abstract

The aromatic profiles of eight white wines from Romanian and international grape varieties were analysed to characterize and also differentiate them. All wines come from the Drăgășani vineyard. Monoterpenes, higher alcohols, fatty acids ethyl esters, acetates were determined by gas chromatography-mass spectrometry (GC-MS). Alcohols were the most abundant class for Sauvignon, Muscat Ottonel varieties, esters for Italian Riesling, Fetească regală varieties. Terpenes (linalool, terpineol, nerol) were identified only in aromatic and semi-aromatic wines - Tâmâioasă românească, Muscat Ottonel and Sauvignon, Fetească regală. According to odor activity values (OAVs), not all of the determined compounds had concentrations higher than their odor thresholds.

***Key words**: wine; aroma profile; volatile compound; Vitis vinifera, GC/MS.*

INTRODUCTION

In the expressiveness and quality of a wine, the aroma compounds play an extremely important role. Wine flavour can be varietal, fermentative or from ageing (Selli et al., 2006).

Wine, being the drink with the most complex aromatic profile, contains approximately 800 aroma compounds. The chemical compounds responsible for wine aroma are mainly alcohols, esters, volatile fatty acids, aldehydes and ketones, of which esters are particularly important (Rapp & Mandery, 1986). In addition to these aroma compounds, it must also be mentioned the terpenols, that contribute decisively to the character and typicality of aromatic wines. Many researchers have studied the volatile composition of different grapes and wines (Gunata et al., 1985; Etievant, 1991; Ferreira et al., 2000; Oliveira et al., 2004; Vilanova & Sieiro, 2007; Vilanova et al., 2013). The quantitative content of volatile compounds in wine can be influenced by several factors, such as grape variety, degree of ripening, climate conditions, fermentation conditions and winemaking practices, and last but not least, aging (Gunata et al., 1985; Rapp, 1998; Heroiu, 1998; Bueno et al., 2003; Oliveira et al., 2004; 2006; Stoica, 2003).

The quantitative analysis of wine aroma can be considered as an important asset for the wine industry, because of the need to have a reproducible analysis to differentiate wines of different varietal origins.

Using chromatographic methods, gas chromatography and especially gas chromatography coupled with mass spectrometry leads to the identification and quantification of aromatic compounds in wine. The predominant compounds identified in wine are monoterpenes, norisoprenoids, alcohols, acids, esters, carbonyls, phenylpropanoids, methoxypyrazines and volatile sulphur compounds (Ferreira et al., 2000; Francis & Newton, 2005).

The aromatic profile of Romanian wines has been studied over the years, but only on specific issues, such as determination of volatile compounds in grapes and wines (Țîrdea, 2007; Băducă-Cîmpeanu, 2016), the aroma profile of traditional Romanian and foreign aromatic varieties (Heroiu, 1998; Stoica, 2015), or the influence of wine processing on the flavour profile (Stoica et al., 2015).

The aim of this work was to define the aromatic profile by determining the major collate compounds of young wines produced from Romanian and foreign varieties (Tămăioasă românească, Fetească albă, Fetească regală, Muscat Ottonel, Sauvignon, Italian Riesling, Chardonnay, Pinot gris) in Drăgășani (Vâlcea) and to identify the most active odour compounds.

MATERIALS AND METHODS

Grape samples

Eight Romanian and international *Vitis vinifera* white grape varieties (Tămâioasă românească, Fetească albă, Fetească regală, Muscat Ottonel, Sauvignon, Italian Riesling, Chardonnay, Pinot gris) from the Drăgășani -Vâlcea vineyard were considered in this study. The different varieties, from the 2021 harvest, were grown under the same conditions in the Drăgășani vineyard.

The Drăgăşani vineyard extend on the right bank of the Olt, to the west and north of the town of Drăgăsani and to the south of Râmnicu Vâlcea, occupying the hills of the Piedmont area of the lower Olt and its main affluents, from the territory of Vâlcea and Olt counties.

The vineyard includes areas of major importance in the national viticulture. Located in the great geomorphological unit Getic Piedmont, the Drăgășani vineyard is located at this coordonate 44°39′40″N latitude and 24°15′38″E longitude, totaling 8,000 ha, at an altitude of 182 m. It is part of the wine-climatic zone A3, which includes regions and wine-producing centers mainly of high-class white, aromatic and red wines and secondary white and red wines for current consumption (Teodorescu et al., 2021).

The wines analysed in this study were obtained in the Oenology laboratory, Faculty of Horticulture, Craiova. The wines were produced in 25 L glass vessels. Before fermentation, sulphur dioxide (4 g/hL) and ascorbic acid (5 g/hL) were added to musts. The wines were elaborated using aromatic winemaking practices, which involves a period of maceration on the lees to favour the extraction of terpenes. For this purpose, a 36-hour maceration was applied, in the presence of the enzyme preparation Zymovarietal aroma G, in a dose of 3 g/100 L. After pressing, the fermentation was conducted using a strain of active selected yeasts (LSA) from the Institute Œnologique de Champagne - *Saccharomyces cerevisiae* var. *bayanus*, IOC-18-2007 (20 g/hL) at 18°C. After fermentation, the wines were filtered and transferred into 0.75 L bottles. The bottles were corked and stored at 16°C until analysis.

Reagents

The chemical standards for GC were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and Fluka (Buchs, Switzerland), having an average purity above 99.5%. Synthetic wine was made using calibration solutions. Synthetic wine consists of 12% v/v ethanol, 5 g/L tartaric acid, brought to 3.3 pH with 0.1N NaOH solution. As internal standard was used 2-Octanol. For each compound were prepared 5-points calibration curves for quantification. Regression coefficients of the calibration curves were above 98%

Volatile compound extraction and analysis

Volatile compounds of the wine samples were liquid-liquid extracted and analysed using gas-chromatography coupled with mass spectrometry (GC/ MS). 100 mL of wine sample was extracted 3 times with 10/5/5 mL of dichloromethane, at 600 rpm, for 20 min. The organic extract was dried and then, concentrated to a volume of 1.5 mL. Carbonyl compounds were determined with PFBOA (ortho-2,3,4,5,6-pentafluorobenzyl-ortho-hydroxil-amine) (Țîrdea, 2007).

The GC/MS used was a Varian 450GC/240MS. The column was a 60 m x 0.25 mm CPSil88, with 0.39 mm film thickness. The carrier gas was helium at a flow rate of 0.9 mL/min. 1 µL of sample was injected in the GC. The oven's starting temperature was 50°C, held for 2 min, then raised to 190°C, at a rate of 3°C/min and held at 190°C for 1.33 min. The mass spectrum in full mode electron impact at 70eV were recorded in the m/z range of 30 - 150. The volatile compounds were identified using standard compounds, mass spectra, mass spectra library and retention times (Milo, 2003; Popescu et al., 2014). Analyses were carried out in duplicate.

Odour activity value

The odour activity value (OAV) was determined to evaluate the contribution of a chemical compound to the aroma of a wine. OAV is a measure of the importance of a specific compound to the odour of a sample. If

OAV > 1 then indicates possible contribution to the wine aroma. This was calculated as the ratio between the concentration of the individual volatile compound and the perception threshold found in the speciality literature (Etievant, 1991; Francis & Newton, 2005; Vilanova et al., 2009; Popescu et al., 2014).

RESULTS AND DISCUSSIONS

The results regarding the concentration of volatile compounds from the 8 white wines, semi-aromatic and aromatic, are presented in Table 1. There were identified 20 different compounds: 3 terpenes 4 alcohols, 5 esters, 5 acids, and 3 aldehydes.

Monoterpenes

Terpenes are constituents of grapes that are in a free and bound state and whose biosynthesis begins with acetyl-coA (Yang et al., 2019). The bound form is not volatile and has a glycoside group, such as glucose, arabinose, rhamnose and apiose (Korenika et al., 2018). The grape varieties also contain β-glucosidase which, under normal winemaking conditions, can release the terpene compounds from the bound forms. This process is accentuated if maceration is used in the vinification of grapes. Therefore, besides the nature of the grape variety and the degree of ripening, the winemaking process plays an important role in the final terpene content of the wines. Terpene compounds such as α-terpineol, linalool, geraniol and nerol are volatile and responsible for the floral notes of the semi-aromatic grape varieties (Sauvignon and Fetească) and grape-aromatic varieties (Tămâioasă românească and Muscat Ottonel), but the distinctive note of the aroma is given both by the concentration in which are found as well as the proportions between them (Lukic et al., 2017).

Some monoterpenes are also found in other grape varieties, which are not usually considered aromatic, but in totally insignificant proportions compared to the aromatic varieties (Riesling Italian, Pinot gris and Chardonnay) (Luan et al., 2006; Oliveira et al., 2008).

In this study, the wines obtained from the aromatic varieties of Tămâioasă românească and Muscat Ottonel presented the highest concentrations of terpenes. It should be noted that the proportions of these compounds in the two wines are different. Thus, α-terpineol predominates in Tâmiaoasa românească wine, while in Muscat Ottonel the higher proportion is found in linalool. All three terpene compounds are found in the wines from the semi-aromatic Fetească and Sauvignon varieties, but in much lower concentrations. Also, in all three semi-aromatic wines, α-terpineol predominates quantitatively.

Differences in concentrations of volatile compounds between the samples in this study and the literature values can be justified by the difference between the preparation method, the place of origin for the wine and the harvest year. On the other hand, the qualitative differences regarding the number and type of detected compounds can be explained by the difference between the methods of preparation and analysis (extraction solvent, extraction time and steps, type of chromatographic capillary column, detector, analysis succession etc.) (Popescu et al., 2014).

Alcohols

Alcohols are also produced during the alcoholic fermentation of carbohydrates. With the exception of 2-phenylethanol, which has the aroma of roses (Etievant, 1991), the other alcohols do not have a pleasant contribution to the aroma of wines. 2-phenylethanol is the compound with the highest concentration in all analysed wines. However, from a quantitative point of view, semi-aromatic and aromatic wines stand out (Tămâioasă românească, Muscat Ottonel and Sauvignon). According to research by Quian et al. (2009), this alcohol can also be present in grapes, but mostly it is produced by yeasts during alcoholic fermentation. Isopropanol was identified only in Sauvignon. 1-hexanol was present in almost all the wines, with the exception of Muscat Ottonel in agreement with other researches (Popescu et al., 2014). The composition of alcohols differed both quantitatively and qualitatively between the varieties, Sauvignon having the largest number of alcohols (4), followed by Tămâioasă românească and Riesling Italian (3). All the other varieties have in composition only 2 alcohols. Tămâioasă românească and Muscat Ottonel wines have the highest alcohol concentration, 111 mg/L and 81 mg/l, respectively. Chardonnay wine, considered neutral, also has high alcohol concentrations.

Esters

Esters are the compounds that have an important contribution to the aroma of young wine. Their origin in wine comes from the metabolism of yeast during alcoholic fermentation, but they are found in small quantities also in grapes (Perestrelo et al., 2006). Acetic esters (ethyl acetate and isoamyl acetate) result from the reaction between acetyl-CoA and higher alcohols. Ethyl esters of fatty acids are produced by enzymes during alcoholic fermentation and from acyl-CoA ethanolysis, being formed during the synthesis or degradation of fatty acids (Zhu et al., 2022). Most esters are formed at the beginning fermentation, their concentration varying to a small extent proportion during wine maturation (Popescu et al., 2014).

Ethyl butyrate, ethyl octanoate and isoamyl acetate were present in each variety. The most abundant ester was probably ethyl acetate formed in small amounts during yeast fermentation and in a larger quantity from the intervention of acetic bacteria, especially during the maceration process and maturation in the barrel, when the wine is still in contact with air.

Ethyl acetate is well perceived between 150-200 mg/L with specific aroma; if it exceeds 200 mg/L influences wine quality (Tarko et al., 2008).

Ethyl acetate from the studied wines, between 4.1 (Fetească wines) and 65 mg/L Riesling Italian wine) favourably influences the aroma, the aromatic variety had an ethyl acetate content within limits, of 18 mg/L in the Tămâioasă românească and 29 mg/L in Sauvignon (semi-aromatic).

In the Tămâioasă românească and Sauvignon varieties, all 5 esters were identified and quantified, analysing the largest number of esters, 6 compounds with total concentrations of 21.8 mg/L and 30 mg/L, respectively.

Table 1. Volatile composition (μg/L) of wines from Romanian and international varieties in Drăgășani vineyard

Compounds	Volatile composition (μg/L) of wines							
	Tămâioasă românească	Fetească albă	Fetească regală	Muscat Ottonel	Sauvignon	Riesling italian	Chardo-nnay	Pinot gris
Monoterpenic alcohols								
Linalool	1010.2	160.8	111.5	2287.8	290.2	18.5	ID/NC	ID/NC
α-Terpineol	3130.0	183.4	107.7	995.3	420.1	47.2	ID/NC	13.70
Nerol	420.1	93.0	90.5	380.1	119.0	1.20	-	ND
Alcohols								
1-Propanol	ID	-	-	-	ID/NC	ID/NC	ID/NC	ID/NC
1-Hexanol	1598.1	980.2	968.3	-	1450.1	972.0	1689.0	267.7
2-Phenyl ethanol	95280.0	99021.1	9804.0	81240.0	35710.0	33200.0	10900.0	9469
Isopropanol	-	-	-	-	50.3	-	-	-
Esters								
Ethyl butyrate	410.3	176.8	167.9	462.5	571.2	291.5	276.1	261.2
Ethyl octanate	720.4	871.0	810.4	2091.0	810.0	2390.0	1451.0	1125.9
Ethyl decanonate	332.3	-	-	291.1	210.0	-	560.2	218.8
Ethyl acetate	18528.1	41991.0	41880.2	-	29710.1	65720.0	-	-
Isoamyl acetate	1890.9	1170.2	1140.7	1941.6	1570.2	2716.8	1510.4	1763.6
Isovalerianic acid	-	-	-	-	1870.6	987.3	2070.1	1200.5
Diethyl acetic acid	1.9	-	-	1.8	-	-	1.9	-
Heptanoic acid	4.1	3.9	3.6	4.4	3.7	4.8	4.2	4.2
Dodecanoic acid	29.8	12.8	12.4	29.3	-	34.0	38.2	21.0
Aldehydes								
Acetaldehyde	39100.0	6820.1	6690.0	42600.0	24890.0	5480.0	-	-
Propion-aldehyde	-	63.4	61.9	-	49.1	48.8	-	-
Hexanal	20.3	20.7	19.8	22.9	21.5	23.5	23.8	-

ID/NC - identified but not quantified

Acids

The formation of acids depends on the composition of must and fermentation conditions (Heroiu, 1998). Isovaleric acid is the most abundant with concentrations between 1.2 mg/L and 2.0 mg/L, although it was not identified in all wines. Heptanoic acid is present in all analysed wine samples. Lactic acid was present only in Sauvignon but could not be quantified. The largest acids concentration is present in Chardonnay (2.1 mg/L), Sauvignon (1.8 mg/L), Pinot Gris (1.2 mg/L) and Riesling italian (1.0 mg/L). The wines of Fetească and the aromatic ones of Tămâioasă and Muscat have the lowest concentration of acids. Acids give the wine freshness and a pleasant aroma at concentrations up to 10 mg/L. At concentrations above 20 mg/L, they have a negative effect on the sensory characteristics of the wine (Heroiu, 1998). The reported values for acids in the studied wines are in very good agreement with those reported in the literature.

Aldehydes

Aldehydes are formed in wine following the metabolism of amino acids and from the enzymatic oxidation of unsaturated fatty acids. The aldehyde content of the wines varied between 0.06 mg/L for Chardonnay and 42 mg/L for Muscat Ottonel.

Among the analysed aldehydes, acetic aldehyde registered the highest values 0.54 mg/L and 42 mg/L. Hexanal was not identified in 4 wines Tămâioasă românească, Muscat Ottonel, Chardonnay and Pinot gris. Aldehyde concentrations from the studied wines are similar to those reported in the specialized literature.

Odour activity value (OAV)

To evaluate the influence of the analysed volatile compounds on the general aromatic profile of wines, the odour activity value (OAV) was calculated by dividing the concentration of each compound by its perception threshold. Only compounds with an OAV greater than 1 individually contribute to wine aroma (Guth, 1997). However, studies by Francis and Newton (2005) showed that when the OAV of a certain compound is less than 1, it can still contribute to the aroma of a wine due to the additive effect of similar compounds (similar structure or smell). The odour descriptor, OAV and threshold for each analysed compound are listed in Table 2.

Table 2. Odour activity values (OAV) in varietal wines

Volatile compounds	Descriptor	Threshold µg/L	OAV							
			T.R.	F.A.	F.R.	M.O.	S.	R.I.	CH.	P.G.
Linalool	Floral, citrus	25.0	40.4	7.33	4.46	91.51	11.60	0.72	-	-
α-Terpineol	Pine, lilac	340	9.20	0.47	0.31	2.92	1.52	0.13	-	-
2-Phenyl ethanol	Rose, perfume	12000	7.94	0.82	0.81	6.77	2.97	2.76	0.90	-
Ethyl butyrate	Pineapple	20.0	20.5	8.79	8.39	23.12	28.56	14.57	13.80	13.06
Ethyl octanate	Pineapple, pear, floral	2.0	360.2	435.5	405.2	1045.5	405.0	1195.0	725.5	562.9
Ethyl decanonate	Grape, pleasant	200	1.66	-	-	1.45	1.05	-	2.8	1.09
Ethyl acetate	Fruity, sweet	75000	0.24	0.55	0.55	-	0.39	0.87	-	-
Isoamyl acetate	Banana	30.0	63.03	39.0	38.0	64.72	52.34	90.56	50.34	58.78
Isovalerianic acid	Fatty, rancid	33.0	-	-	-	-	54.77	29.91	62.73	36.37
Acetaldehyde	Ethereal, fruity	65000	0.60	0.10	0.10	0.65	0.38	0.08	-	-
Propion-aldehyde	Whiskey, nutty	24.0	0.84	0.86	0.82	0.95	0.89	0.97	0.99	-
Hexanal	Fresh, green	4.7	-	13.48	13.17	-	10.44	10.38	-	-
TOTAL										

Descriptors and flavour thresholds of volatile compounds found in the literature (Etievant, 1991; Ferreira et al., 2000; Francis & Newton, 2005; Vilanova et al., 2009)

Nine volatile compounds out of 20 analysed (45%), identified in the wines presented an OAV > 1, contributing to the aroma of the analysed wines. And other authors (López et al., 1999; Escudero et al., 2004; Jiang & Zhang, 2010) showed similar results in other young white and rosé wines. Ethyl-butyrate, isoamyl acetate, ethyl octanoate contributed to the wine aroma of all studied varieties. The floral character was represented by three compounds with OAV > 1. Linalool only contributed to the floral character of the wines Tămâioasă romanească, Fetească albă, Fetească regală, Muscat Ottonel and Sauvignon, α-terpineol in Tămâioasă românească, Muscat Ottonel and Sauvignon wines and 2-phenylethanol seemed to contribute only to the same character in Tămâioasă românească, Italian Riesling and Sauvignon wines, as the concentrations were above the odour threshold.

Ethyl decanoate, ethyl butyrate and isoamyl acetate also made an important contribution to the aroma of all monovarietal wines.

The most intense odorants in Tămâioasă românească and Muscat Ottonel wines produced in Drăgășani vineyard are linalool, α-terpineol, 2phenyl-etanol, ethyl butyrate, ethyl octanate and isoamyl acetate.

CONCLUSIONS

The formation of volatile compounds in wines is complex process and depends on the chemical composition of the grapes and the maceration and fermentation process.

The main aim of this study was to characterize and differentiate wine varieties according to their volatility composition (flavour profile). It was found that some wines they are more aromatic than others. Tămâioasă românească, Muscat Ottonel and Sauvignon are the most aromatic, with the main floral aroma persisting. The compounds that greatly influence the aroma, in addition to the terpenes, are ethyl octanoate, isovaleric acid and isoamyl acetate. The overall aroma of the wine study is dominated by the maceration and fermentation stage, namely by ethyl esters of fatty acids (ethyl esters of butyric, octanoic and decanoic acids), which give the wine fresh, fruity notes. Also, the aroma profile of three Romanian autochthonous wine varieties (Tămâioasă românească, Fetească albă and Fetească regală) was quantified and classified, recording higher values than other international varieties. The results are preliminary, the interpretation provided being limited by the fact that all wines were produced for a single vintage, with no repetitions for variants.

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ESTIMATING THE TOLERANCE OF THREE TABLE GRAPE VARIETIES TO WATER STRESS BY CHLOROPHYLL FLUORESCENCE ANALYSIS

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Abstract

Chlorophyll fluorescence analysis is one of the modern techniques used to study the effect of stress on the photosynthetic process. In our research, we monitored through periodic determinations the index of chlorophyll, the content of the main parameters of chlorophyll fluorescence and photosynthetic potential, in order to determine the thermal resistance threshold at three varieties of table grapes grown in the Stefanesti, Arges County area. All plants analyzed were subjected to three temperature thresholds: 3.3-7.1ºC, 19.4-21.6ºC and 36.5-43.5ºC (increased heat stress). The 'Argessis' variety proved to be the most resistant, followed by 'Victoria', and the 'Augusta' variety was the most affected by high temperatures, with highly significant positive correlations. Very significant positive correlations were found between OJIP indicators and Phi Do indicator, Pearson correlation values ranging from 0.749 to 0.701. These methods are supportive valuable indicators for establishing the tolerance of table grape varieties to water stress, for tailored irrigation management and for an appropriate choose of resilient varieties in the climate change context

Key words*: Chlorophyll fluorescence water deficit, Vitis vinifera L, environmental drought stress, photosystem II efficiency.*

INTRODUCTION

Abiotic stresses are often interdependent, either individually or combined, lead to morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity and ultimately yield. Heat, drought, cold and salinity are the major abiotic stresses that induce severe cell damage in plant species.

Determination of drought tolerance crop plants can be expensive requiring time-consuming and labor-intensive techniques, field tests, etc. A promising approach is the use of chlorophyll fluorescence, a technique that can provide large amounts of data with minimal time and without harming plants. Chlorophyll fluorescence it works on the principle that photosynthesis is one of the basic functions in plant physiology. In the last decade, chlorophyll fluorescence has been widely used to document the physiological effects of plant stress; plants subjected to almost any stress, including heat stress, will show changes in fluorescence (Knight and Ackerly, 2002; Yamada et al., 1996). Baker and Rosenqvist, (2004), Gamon and Pearcy, (1989), state that, under stress conditions, Fv/Fm (where Fv is variable fluorescence and Fm is maximum fluorescence) decreases due to an increase of minimum chlorophyll fluorescence (Fo). In the last decade, chlorophyll fluorescence has been widely used to document the physiological effects of plant stress. Plants subjected to almost any stress, including heat stress, will show changes in fluorescence Fv/Fm ratio (Fv is variable fluorescence and Fm is maximum fluorescence) which decreases due to an increase in of minimum chlorophyll fluorescence (Fo) (Belkhodja et al., 1994;

Bukhov and Carpentier, 2004; Maxwell and Johnson, 2000).The OJIP test was first proposed by Strasser and Strasser (1995), being used to translate the original measurements of transient fluorescence in various phenomenological and biophysical expressions quantifying PSII function (Tóth et al., 2007).

According to the Manual of Opti-Sciences (http://www.optisci.com/cf.htm - Stress Testing), the OJIP test is a rapid, dark-adapted test that uses a high capture rate of signal for analyzing their fluorescence, with an emphasis on the kinetics of the rapid increase in initial fluorescence, using strong actinic light. In the initial phase of the fluorescence increase, the resulting curve was found to show intermediate inflections before reaching FM or P. These intermediate peaks or steps (levels) are designated as J, I and P, starting from O, this being the value of the signal of the initial fluorescence measured after 20 µsec (Strasser, 2004). In addition to the JIP steps, an additional step called K occurs during specific types of stress (Strasser, 2004). According to data from the specialized literature, thermal stress triggers the destruction of the manganese complex of the oxygen-producing complex (Yamane et al., 1998, cited by Tóth et al., 2006), which has the role of splitting water, the source of electrons for the transport chain of electrons. The most used parameter in the OJIP test is the PI performance index, an indicator with three main attributes that determine the potential of photosynthetic activity, the density of reaction centers, the probability that an absorbed photon to be used for charge separation (initial electron transfer) before electron transfer. Thermal stress causes significant changes in the fluorescence, including an increase in the initial fluorescence (F0) and a decrease in the maximum fluorescence values (FM). An additional peak at approximately 0.3 ms can also be observed, which is called band K (Oukarroum et al., 2012 Weng and Lai, 2005).

The objective of this study was to evaluate the effects of different temperatures on the photosynthetic activity of 3 varieties of table grapes in order to determine the resistance threshold to drought. The chlorophyll index, changes in chlorophyll content that can occur as a result of plant exposure to environmental stress, was also assessed.

MATERIALS AND METHODS

The study has been carried out in 2022, in a table grape vineyard, located at the National Research and Development for Biotechnology in Horticulture Ștefanești, Argeș County (NRDIBH Stefanesti). The region is characterized by a humid temperate-continental climate, with a mean annual temperature (T. mean) of 9.6°C and a precipitation amount of 671.8 mm for the 1979-2020 period not uniformly distributed across the year. The large amplitude of meteorological conditions occurring during the vegetation period from 2022 at NRDIBH Stefanesti, has the specificity of an excessively continental climate, this year's vegetation season being a very dry one (only 530 mm compared to the multi-year average of 671.8 mm). The driest months were May, June and October, with precipitation of only 16.2 mm, and 3.6 mm, respectively 7.2, the number of rainy days being 4-9. In the air, the last minimum temperature was recorded in April (-2.8ºC) and the maximum temperature in July 36.08ºC.

Determination of the chlorophyll index (CCI)

In our study, in August, we monitored the influence of the 3 varieties of table grapes (‘Augusta’, ‘Victoria’, and ‘Argessis’) and the position of the plants along the watering tubes through 3 periodic determinations of the chlorophyll content index with CCM-200 OPTI-SCIENCES device. The measurements were performed on young leaves, in the phenophase of plant’s growth (Filimon et al., 2014). For each experimental variant, 30 determinations were performed from a bifactorial scheme, as follows:

- factor A, table grape variety with 3 gradations:
- a_1 - ‘Augusta’,
- a_2 - ‘Victoria’,
- a_3 - ‘Argessis’;
- factor B - the position of the plants along the watering tube:
- b_1 - I-Third location - the analyzed plant in the first third of the watering tube, where it is assumed that the supply of water is richer;

- b2. - II third location - in the middle of the watering tube and
- b_3 - III- third location - in the last third of the watering tube.

Determination of the OJIP chlorophyll fluorescence indicator

Three leaves from each variety ('Augusta', 'Victoria' and 'Argessis') were detached on August 12th, quickly placed with the base in water in Erlenmayer dishes and adapted to the dark and passed through three times successively through three temperature thresholds: 3.3-7.1ºC, 19.4-21.6ºC and 36.5-43.5ºC (accentuated thermal stress).

Using the OJIP test, determined with the FP110 fluorometer, 25 indicators were calculated at each temperature change (14 determinations), as follows:

Fo = F50 µs; chlorophyll fluorescence intensity at 50 µs (initial);

Fk = fluorescence intensity at level k (at 300 µs);

Fj = fluorescence intensity at level j (at 2 ms);

Fi = fluorescence intensity at level i (at 60 ms);

Fm = maximum fluorescence intensity;

Fv = Fm - Fo - maximum fluorescence amplitude;

Vj = (Fj - Fo)/(Fm - Fo) - the relative fluorescence amplitude at level j, for non-connected FS II units;

Vi = (Fi - Fo)/(Fm - Fo) - the relative fluorescence amplitude at level i;

Fm/Fo = ratio of maximum to minimum (initial) fluorescence;

Fv/Fo = capture probability performance ΦPo (PTR) [ΦPo/(1 - ΦPo)], contribution to the performance index (PI) of light-driven reactions for primary photochemical centers. The contribution of light-produced reactions to primary photochemistry is estimated according to the JIP test as [ΦPo/(1 - ΦPo)] = Fv/Fo;

Fv/Fm = quantum potential yield (efficiency) of photosystem II (FS II), in a dark-adapted leaf. It is an indicator of the FS II integrity of the plant. A healthy land plant will almost always have an Fv/Fm value close to 0.8. A decrease in the ratio value will indicate the presence of stress conditions and a fluorescence quenching mechanism;

Mo or (dV/dt)0 = TRo / RC - ETo / RC = 4 (F300 - Fo) / (Fm - Fo) – initial approximate slope (in ms-1) of transient fluorescence V = f(t); FS II net closing rate: (dV/dt) or Mo = 4 (F300µs – Fo)/(Fm – Fo).

Area = the area between the fluorescence curve and Fm (subtract the starting level).

Fix Area = The total area above transient OJIP fluorescence - between F40µ and F1s (subtract the starting level);

Sm = Area/Fm - Fo ("turn-over" multiple);

Ss = the smallest Sm turn-over (a single "turn-over" - inflection of the transient curve);

N = Sm . Mo . (1/Vj) – number "turn-over" QA (QA reduction through FS II activity).

Production or Quantum efficiency or electron flux ratios:

Phi_Po (ΦPo) = 1- (Fo/Fm) (or Fv/Fm) = the maximum quantum yield of primary photochemical reactions at t=0.

Psi_o (Ψo or ETo/TRo) = 1 - Vj = the probability (at time 0) that an excited donor transfers an electron in the electron transport chain beyond QA (the primary acceptor);

Phi_Eo (ΦEo) = (1 - Fo/Fm)). Psi_o - quantum yield for electron transport at t = 0;

Phi_Do (ΦDo) = 1 - Phi_Po - (Fo/Fm) - the quantum yield at t = 0 for energy dissipation;

Phi_Pav (ΦPav) = Phi_Po - (Sm/tFm); (tFm) = rise time at Fm (ms);

Pi_Abs = absorption-based performance index (PI).

Specific flows or activities expressed per reaction center (RC):

ABS/RC = Mo . (1/VJ) . (1/Phi_Po);

TRo/RC = Mo . (1/Vj) - energy flow captured on the reaction center (RC) la t=0;

ETo/RC = Mo . (1/Vj) . Phi_o) - the flow of electrons carried on the RC at t=0.

Relations between chlorophyll content and photosynthesis variables were tested, with linear regression analysis, and Pearson correlation coefficients. Analysis of variation (ANOVA) and Duncan's test were performed to test the mean differences between experimental factors.

RESULTS AND DISCUSSIONS

Maximum fluorescence (FM) showed significant decreases starting from 36.4°C, where the lowest values were recorded. (Table 1, Figure 1). Temperature thresholds significantly affected Fo and Fm, which resulted in significantly different values of Fv/Fm ratio. This was true for all three varieties studied. The Fv/Fm measurements had values between 0.80 and 0.61, after reaching the threshold of 36.4ºC. A healthy land plant will almost all the time have an Fv/Fm value close to 0.8 (Tóth et. al., 2007). A decrease in the ratio value will indicate the presence of stress conditions and a fluorescence quenching mechanism (Tóth et. al., 2007). Analyzing this indicator, at the temperature threshold 36.5-43.5ºC, from table 2 it can be seen that the highest values were recorded in the 'Augusta' variety, and the lowest in the 'Argessis' variety, the latter suggesting a greater tolerance to water stress. The 'Victoria' variety recorded intermediate values of FO (421), at the same temperature threshold analyzed. Bussotti et al. (2011) in their research have affirmed that an F0 increase can be interpreted as indicative of irreversible damage to PSII caused by uncontrolled heat dissipation that produces an excess of excitation energy. Fo values can increase when there is a slowdown in excitation energy transfer from the light collection system to the reaction center (Baker and Rosenqvist, 2004), or when there is some type of damage in the PSII reaction centers themselves (Vieira et al., 2010). This parameter represents the number of open reaction centers, or, rather, the first electron acceptor of PSII, QA, in its oxidised state. (Bussotti et al., 2011; Strasser et al., 1995). However, the higher values of the Fv/Fm ratio in the 'Argessis' variety, closely followed by the 'Victoria' variety, measured at a temperature of 43.5ºC (0.64 and 0.63, respectively), compared to only 0.58 recorded in the 'Augusta' variety, indicates greater tolerance of plants to higher temperatures (increased heat stress) (Figure 1). The flux of absorption and trapping per reaction center (RC) of PSII, defined as ABS/RC, (RC) of PSII, ABS/RC, and TR_0/RC, respectively, were significantly higher in leaf discs incubated above 36.5ºC (Table 1). The specific fluxes expressed per reaction centers (ABS/RC; TR_O/RC; DI_O/RC; ET_O/RC) were derived from Sironval's theory of energy flow through biomembranes and were calculated using the OJIP test (Strasser et. al., 2001).

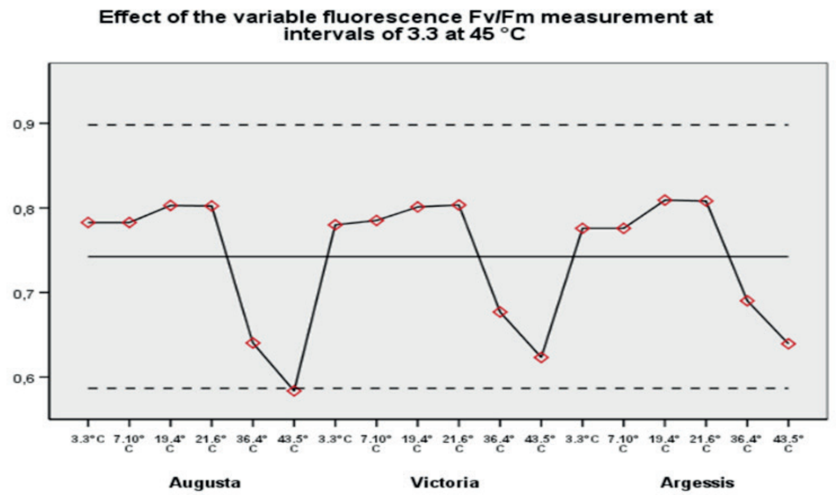

Figure 1. Variation of the OJIP - Fv/Fm indicator according to the combinations of gradations of the 2 experimental factors (Variety and Temperature, NRDIBH Stefanesti, 2022)

In our study, the ABS/RC indicator recorded the highest values in the 'Augusta' variety (7.53) after exposing the plants to the threshold of temperature by 36.5-43.5ºC, compared to only 6.40, respectively 6.45 that were recorded in the 'Argessis' and 'Victoria' varieties, at the same temperature threshold analyzed. The lowest values of ABS/RC were evident at the temperature threshold of 3.3-7.1ºC, in all the varieties analyzed, with values between 1.47

and 1.49 (Table 1). The term absorption (ABS) refers to the absorption of photons by chlorophyll molecules in antenna-like complexes. The electron transport flux per RC of PSII defined as ET_0/RC started to increase at the temperature of 36.5°C. It was not possible to estimate the reduction in terminal electron acceptor flux (RE_0/RC) for temperatures above 36.5°C due to the change in the shape of the kinetics of the fluorescence emission curve (Table 1). These statements are also supported by Chen et al. (2008) in selling research. Therefore, the intervals 3.3-7.1ºC were chosen; 19.6-21.4ºC, respectively 36.5-43.5ºC, between these thresholds there are no differences between the measurements (Table1).

Table 1. Chlorophyll fluorescence intensity of the OJIP assay parameters from the fluorescence transient at three table grapes subjected to different temperatures

		Intensity chlorophyll a fluorescence				
Variety	**T (ºC)**	**To**	**Tm**	**Fm/Fv**	**Fv/Fo**	**Fi**
‘Augusta’	3.3-7.1ºC	315^{b}	1450^{ab}	0.78^{a}	487^{b}	3.60^{ab}
	19.6-21.4⁰C	336^{b}	1700^{a}	0.80^{a}	641^{a}	3.90^{a}
	36.5- 43.5ºC	454^{a}	$1175{,}5^{b}$	0.61^{b}	571^{a}	1.59^{b}
‘Victoria’	3.3-7.1ºC	332^{b}	1525^{ab}	0.78^{a}	431^{b}	$3.6a^{b}$
	19.6-21.4⁰C	339^{b}	1715^{a}	0.80^{a}	641^{a}	4.06^{a}
	36.5- 43.5ºC	421^{a}	1210^{b}	0.65^{b}	645^{a}	1.87^{b}
‘Argessis’	3.3-7.1ºC	325^{b}	1450^{ab}	0.78^{a}	495b	3.46^{ab}
	19.6-21.4⁰C	330^{b}	1725^{a}	0.80^{a}	641a	4.22^{a}
	36.5-43.5ºC	415^{a}	1245^{c}	0.66^{b}	654a	2.00^{b}

		Flux per reaction centers		
Variety	**T (ºC)**	**ABS/RC**	**TR_0 /RC**	**ET_0 /RC**
‘Augusta’	3.3-7.1ºC	1.48^{b}	1.17^{b}	0.38^{b}
	19.6-21.4⁰C	1.62^{b}	1.20^{b}	0.61^{a}
	36.5-43.5ºC	7.53^{a}	3.28^{a}	-
‘Victoria’	3.3-7.1ºC	1.47^{b}	1.20^{b}	0.31^{b}
	19.6-21.4⁰C	1.56^{b}	1.21^{b}	0.58^{a}
	36.5-43.5ºC	6.43^{a}	3.15^{a}	-
‘Argessis’	3.3-7.1ºC	1.49^{b}	1.18^{b}	0.29^{b}
	19.6-21.4⁰C	1.58^{b}	1.20^{b}	0.58^{a}
	36.5-43.5ºC	6.40^{a}	3.08^{a}	-

∗Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$.

In order to test the effect of thermal thresholds on the 26 OJIP indicators measured with the FP 110 fluorometer, the intensity of linear correlations was established using the simple Pearson correlation coefficient (r), and their statistical significance was established (Sig.), after which the OJIP indicators were ordered in ascending order by "r" values (Table 2). It can be seen that the OJIP indicators are most affected by the increase in air temperature (lower and lower values with the increase in air temperature in the range of 3.3-43.5ºC), having the lowest values of the simple correlation coefficient (in the conditions where the significance correlation statistic was highly significant), were in order: Ss (lowest Sm - transient curve inflection) with r = -0.704ººº, Fv/Fm (quantum potential yield of photosystem II - FS II) whose optimal value is 0.8) and Phi_Po (maximum quantum yield of primary photochemical reactions at t = 0. Thus, the correlation coefficient for the maximum quantum yield of primary photochemical reactions (Phi_Po) has a value of r = -0.692ººº, and for Fm/Fo (ratio between maximum and minimum fluorescence) together with Fv/Fo (contribution to the performance index). (PI) of the reactions carried out in the light) a correlation coefficient was found with r = -0.628ººº, respectively r= -0.626 ººº. At the opposite pole, with highly significant positive correlations (the increase of temperature increased the values of the respective indicators), the OJIP indicators of energy dissipation: the specific fluxes or activities expressed on the reaction centers (ETo/RC, TRo/RC, DIo/RC and ABS/ RC), and Phi_Do which represents the quantum yield at t = 0 for energy dissipation. Pearson correlation coefficient values ranged from 0.749 to 0.640

(Figure 2). The lower values of PI_ABS recorded at high temperatures to which the plants were subjected, may be caused by the absorption of energy by inactive reaction centers, which results in lower values of a maximum quantum yield of primary photochemical reactions at t = 0 (Phi_Po) and a reduction in yield of Phi_Eo electron transport (Table 2). Similarly, Zushi et al., 2012, found that specific fluxes per RC increase with temperature in most horticultural species. The increase in ABS/RC indicators, TRo/RC and DIo/RC on active RC was observed due to the inactivation of more RCs, which also suggests an increase of the total dissipation ratio to active RCs due to the high dissipation caused by the RCs inactive.

Table 2. The intensity of the correlation between air temperature and OJIP indicators of chlorophyll fluorescence of the three table grapes varieties

Chlorophyll fluorescence indicator	Simple Pearson correlation indicator	Sig. (2-tailed)	Symbol of statistical significance
Ss	-0,704	0,000	ooo
Fv/Fm	-0,692	0,000	ooo
Phi_Po	-0,691	0,000	ooo
Fm/Fo	-0,628	0,000	ooo
Fv/Fo	-0,626	0,000	ooo
Pi_Abs	-0,462	0,000	ooo
Fv	-0,458	0,000	ooo
Area	-0,422	0,000	ooo
Fm	-0,389	0,000	ooo
Fi	-0,344	0,000	ooo
Fix Area	-0,337	0,000	ooo
Vi	-0,287	0,000	ooo
Phi_Eo	-0,265	0,001	oo
Fj	-0,240	0,003	oo
Vj	-0,201	0,020	o
Sm	-0,161	0,058	
Psi_o	0,191	0,018	*
N	0,261	0.001	**
Fo	0,328	0,000	***
Phi_Pav	0,447	0,000	***
Mo	0,431	0,000	***
Phi_Do	0,64	0,000	***
ETo/Rc	0,658	0,000	***
TRo/RC	0,72	0,000	***
Dio/RC	0,736	0,000	***
ABS/RC	0,744	0,000	***

Analyzing Figure 2, we observe both the main effects of the 2 experimental factors, as well as the interactions A (variety) x B (plant position on the watering tube row) generated by them on the chlorophyll index values. It can be seen that the 2nd and 3rd positions on the watering tube provided a significant increase in CCI compared to the 1st third (Figure 2).

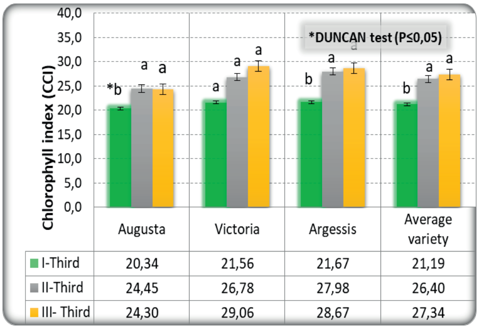

Figure 2. The influence of the position of the watering tube on the chlorophyll index of the three table grapes varieties

Table 3. The intensity of the correlation between the leaf chlorophyll index (CCI) and the OJIP indicators of the chlorophyll fluorescence of the three table grapes varieties (NRDIBH Stefanesti, 2022)

Chlorophyll fluorescence indicator	Simple Pearson correlation indicator	Sig. (2-tailed)	Symbol of statistical significance
Mo	-0,478	0,000	°°°
Vj	-0,457	0,000	°°°
Fo	-0,271	0,000	°°°
Phi_Do	-0,269	0.001	°°°
Fj	-0,256	0,001	°°°
DIo/RC	-0,201	0.020	°°
ABS/RC	-0,178	0,025	°
Vi	-0,177	0,026	°
TRo/RC	-0,156	0,048	°
N	-0,04	0,678	
Fi	-0,037	0,710	
Phi_Pav	0	1,000	
Phi_Eo	-0,265	0,001	
ETo/Rc	0,008	0,917	
Sm	0,051	0,572	
Fix Area	0,074	0,263	
Fm	0,105	0.221	
Area	0,114	0,185	
SS	0,209	0,018	*
Fv/Fm	0,256	0,001	***
Phi_Po	0,257	0,001	***
Fv/Fo	0,316	0,000	***
Psi_o	0,316	0,000	***
Phi_Eo	0,578	0,000	***
ABS/RC	0,585	0,000	***

The investigations carried out in 2022 aimed at verifying the studied varieties, their behavior under the excessive continental climate and irrigation efficiency measures applied especially to overcome water stress. This has been done in particular by analyzes of CCI chlorophyll index dynamics, chlorophyll fluorescence and photosynthetic potential, taking into account 26 OJIP parameters. The chlorophyll content index is highly positively correlated significantly with indicators of chlorophyll fluorescence - OJIP, of quantum production or efficiency, ABS/RC, Psi_Eo, Psi_o, Phi_Eo, Phi_Po, but also with Fv/Fm the quantum potential of IInd photosystem (Table 3).

CONCLUSIONS

From our research, it was found that there was a positive relationship between Fv/Fm ratio and temperatures. As the temperature increased, after 36.5°C, Fv/Fm ratio decreased greatly, indicating a decrease in the photochemical efficiency of PSII.

The Fv/Fm ratio and the valuable OJIP indicators can still be useful for determining the threshold between moderate and excessive water deficit in vines. The higher values of the Fv/Fo, Fv/Fm ratio and absorption flux per reaction center (RC) of PSII, defined as ABS/RC, ET_0/RC and TR_0/RC, respectively, in 'Argessis' and 'Victoria' varieties, measured at 36.5-43.5°C, indicate a greater tolerance of the plants to higher temperatures (increased thermal stress).

These varieties are recommended to be further studied by reducing the water supply in a controlled manner within the vineyard, being an important tool for a moderate level of water deficit that allows the plants to maintain the functions of the leaves and the entire vegetative system, as well as table grape quality indicators.

Highly significant positive correlations were found between temperature and OJIP indicators of energy dissipation (ETo/RC, TRo/RC, DIo/RC and ABS/RC), as well as Phi_Do. Thus, the increase of temperature led to increase the values of the OJIP indicators of energy dissipation and Phi_Do, the values of the Pearson correlation coefficients ranging between 0.749 and 0.640.

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IMPACT OF *PLATANUS* L. SPECIES ON THE POLLEN EMISSIONS AND AIR POLLUTION OF SOFIA

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Abstract

In the last decades, the presence of Platanus species in the green infrastructure of Sofia has increased. The species are quite tolerant to urban conditions, which makes them preferable for landscaping. Their increased quantity leads to a risk of increased allergenic potential.
The aim of this research was to establish the dynamics of Platanus L. pollen concentrations and to assess their relation to meteorological variables, as well as the impact of plane-tree pollen emissions on the air quality in Sofia.
The analysis of the aeropalynological data on the city of Sofia for the period 2013-2022 found a difference in the length of the flowering period over the 10 years of observation. In general, at the beginning of the studied 10-year period, flowering started in the second half of March, while in 2018 and from 2020 to 2022, it began from the first half of April. The shortest flowering period was in 2015, in contrast to the longest duration's in the following two years (2016 and 2017). In 2016 and 2017, the flowering continued in June and the beginning of July. In 2013, 2014 and 2019 the peak was similarly in the second half of April when temperatures rose and the pollen season was extended to about 75 days.
The results of the study showed that the meteorological factors which directly affect the concentrations of airborne pollen were wind speed, relative humidity, atmospheric pressure and solar radiation.
For the time being, the amount of Platanus pollen in Sofia is relatively low. However, in recent years there has been an upward trend. That growth trend is expected to continue due to the degree of maturity of Platanus specimens. The presence of that species as a dominant element in the urban green infrastructure of Sofia should be reconsidered, not only to improve air quality, but also to enhance urban biodiversity, its resilience and ecosystem services.The obtained correlations between seasonal pollen levels and some particulate matter 10 (PM_{10}) also highlight the possible contribution of Platanus pollen to air quality deterioration in Sofia.

Key words: *Platanus, allergenic pollen, meteorological variables, PM_{10}.*

INTRODUCTION

Platanus L. species are popular urban woody ornamentals cultivated in large numbers as street and park trees all over Europe (Pauleit et al., 2002) and worldwide, in Australia and in North and South America (McBride, 2017), in China (Jing et al., 2020).
The genus consists of fast-growing and long-lived species with high ornamental qualities. Along with their good ecological adaptability to urban conditions and tolerance to air pollution, they have a high capacity to retain particulate matter (Baldachini et al., 2017).
However, the presence of *Platanus* species in the urban green infrastructure is associated with disservices, such as emissions of allergens (Cariñanos et al., 2020; Vrinceanu et al., 2021) and Biogenic Volatile Organic Compounds (BVOCs), both contributing negatively to air quality. *Platanus* spp. are proved to be one of the main urban emitters of monoterpenes and sesquiterpenes, which are BVOCs participating in the formation of atmospheric ozone (Xiaoshan et al., 2000; Curtis et al., 2014, Jing et al, 2020). Moreover, the London plane has an Allergenic Potential Value of 24 (VPA), one of the highest in the database of parameters for the calculation of the Index of Allergenicity of Urban Green Zones (IUGZA) (Cariñanos et al., 2019). According to Magyar et al. (2022) *Platanus* x *acerifolia* (Aiton) Willd. falls within the category of taxa having very high potential allergenicity under an evidence-based categorization (CARE-S), developed using genetically determined factors of plants, such as immunogenicity, morphology, and pollen production. Consequently, the authors do not recommend this species for further planting in public urban green areas.
Platanus spp. pollen triggers allergies in the Mediterranean region: Spain (Alcázar et al.,

2015; Cariñanos et al., 2020) Italy (Bedeschi et al., 2007; Cipriani et al., 2019), Portugal (Loureiro et al., 2005; Ribeiro and Abreu, 2014) and Greece (Gioulekas et al., 2004), as well as in Iran (Sedghy et al., 2017) and China (Jing et al., 2020).
The estimated large amounts of pollen production, 3.3×10^6 pollen grains per inflorescence (Maya-Manzano et al., 2017), indicate *Platanus* as one of the biggest pollen emitters among woody anemophilous species (Damialis et al., 2011).
The current phenological dynamic has been altered by the microclimatic parameters of the environment which led to the earlier onset, longer season and more intense flowering, as well as to large quantities of pollen emitted by a tree (Alcázar et al., 2015; Cariñanos et al., 2020).
A long-term (1982-2015) aerobiological study that took place in Brussels associated the overall increasing trend in daily airborne pollen concentrations and an earlier onset of the flowering period for *Platanus* spp. with the rates of change in the annual cycles of various meteorological variables, air temperature, solar radiation, relative humidity, and precipitation (Bruffaerts et al., 2018).
Over 3,200 inventoried specimens were mapped in the Sofia Municipality Green Areas Registry (ROPKR). The analysis of the dendrological composition of urban street tree plantings in Sofia (Anisimova, 2023) indicated the dominant presence of *Platanus* x *acerifolia* (15.09%) in street landscaping, comprising mainly of young specimens (DBH < 25).
The results from the pilot study of sensitization of patients with pollinosis in Sofia (Nikolov et al., 2021) showed that, at present, the sensitization to pollens of different *Platanus* species is not comparable to that to grass or birch pollen. However, having in mind that mature trees with bigger crown volumes produce substantially more pollen than younger trees, along with other modified environmental factors in the era of climate change, *Platanus* pollination disservices with an impact on human health are expected to increase in the future (Cariñanos et al., 2020).
There is evidence that the allergic response in sensitized patients exposed to high or very high pollen concentrations in the air is very intense and further exacerbated by co-factors, such as particular weather variables or high concentrations of atmospheric pollutants (Oduber et al., 2019; Sauliene et al., 2019; Cariñanos et al., 2021). Weather variables substantially influence pollen concentration, with air temperature, solar radiation and relative humidity being the most significant factors (Rosianu et al., 2022).
The aim of this research was to establish the dynamics of *Platanus* L. pollen concentrations and to assess their relation to meteorological variables, as well as the impact of plane-tree pollen emissions on the air quality in Sofia.

MATERIALS AND METHODS

Study area

The city of Sofia (42.70°N 23.33°E) with an altitude of about 550 m is situated in the western part of Bulgaria, in Sofia Valley, bordering on the south with Vitosha mountain. According to Stanev et al. (1991) the climate of Sofia is continental.
The city of Sofia occupies an area of 492 km^2, with a population of about 1 248 452 citizens (NSI, 2022).

Platanus pollen data

The three species cultivated in the green infrastructure of Sofia are *Platanus orientalis* L., *Platanus occidentalis* L. and *Platanus* x *acerifolia* (Aiton) Willd.
The Durham gravimetric method for aero sedimentation of pollen grains on a film-coated microscope slide was used for obtaining the aerobiological data. The pollen trap was installed at 10 m above the ground. The aerobiological monitoring period started at the beginning of March 2013 and lasted till the end of June 2022. The microscope slide was replaced after 24-hour deposition period and daily airborne pollen counts were conducted. The microscopic identification and counting of pollen grains were performed manually on a 3.24 cm^2 surface of the slide.

Meteorological data

The data series for the hourly values of the studied meteorological variables: air temperatures (°C), relative humidity (%), wind speed (m/s) and wind direction, atmospheric pressure (mbar), solar radiation (W/m^2), for the period 2013-2022 were provided by the Bulgarian Executive Environment Agency (ExEA).

Air pollution data

The data series for the hourly concentrations of PM_{10} for the period 2013-2022 were provided by ExEA.

Statistical analysis

Mean values of the hourly averages for the meteorological variables and mean values of the hourly averages for particulate matter 10 (PM_{10}) concentrations were calculated for 15-day periods. Descriptive statistics for meteorological variables and PM_{10} concentration and coefficients of variation (CV) were calculated.

Non-parametric Spearman's analysis with significant correlations ($p < 0,05$) between aeropalynological data and meteorological and air pollution data were tested in order to identify significant relationships between variables.

RESULTS AND DISCUSSIONS

The analysis of the aeropalynological data for the city of Sofia found a difference in the length of the flowering period over the 10 years of observation (Figure 1). The shortest flowering period was in 2015, in contrast to the longest durations in the following two years (2016 and 2017). In 2016 and 2017, the flowering continued in June and the beginning of July. In general, at the beginning of the studied 10-year period, flowering started in the second half of March, while in 2018 and from 2020 to 2022, it began from the first half of April.

In comparison, the *Platanus* pollen season in Poznan started at the turn of April and May and ended usually in the third decade of May, according to an aerobiological study for the period (2005-2009) (Nowak et al., 2012). The aerobiological study of this taxa in the atmosphere of the city of Granada showed that the pollen season start date took place from the beginning to the end of March, and the pollen season end date took place from mid-April to the end of May, with a peak in mid-March (Cariñanos et al., 2020). *Platanus* L. has been reported to be a good bioindicator of temperature variations in previous studies on the impact of climate change on *Platanus* pollination in different European bioclimatic zones (Tedeschini et al., 2006).

Some authors report similar matches between the pollen peaks and periods with lack of precipitation, low relative humidity, respectively, and increased temperatures (Álvarez-López et al., 2022).

The comparison between the peak of the *Platanus* pollen season and the mean amount of pollen grains for the 10-year data series found matches in 2013, 2015, 2018, 2019, and 2022. In contrast to the aforementioned 5 years, in 2014, 2016, and 2017, the peak in the pollen season was 15 days earlier than the average for the 10-year period. In 2020, two pollen peaks were established, in the first half of April and May, respectively, in the context of gradually rising air temperatures. In 2021, the peak pollen release phase was delayed by 15 days and occurred at the highest mean air temperature values reported for the entire pollen season.

The annual pollen concentrations and the daily pollen peaks vary considerably over the years. The probable causes for these fluctuations could be attributed to the accelerated growth or mortality of trees or different pruning approaches (Cariñanos et al., 2020).

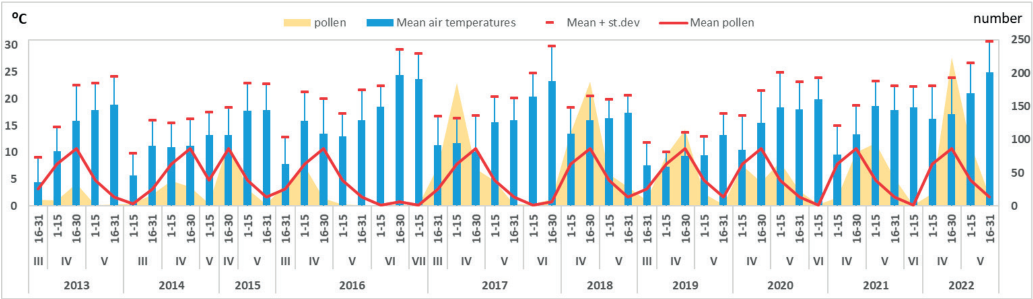

Figure 1. Dynamics of the mean values of the hourly averages of air temperatures and the amount of pollen in the flowering phase over the period 2013-2022

Temperature has been the weather parameter with the greatest impact on the *Platanus* pollen season (Tedeschini et al., 2006; Maya-Manzano et al., 2017).

Within the pollen season, over the 10-year data series, 2014 was found to be the coolest, with a mean temperature in the first half of May of only 13.2°C, in contrast to 2022, which was the warmest for the studied period with a mean temperature of 24.9°C reached in the second half of May (Figure 1).

For the approximately short 60-day flowering period in 2018 and 2022, the maximum amounts of pollen in the air were recorded when temperatures rose in the second half of April. In 2013, 2014, and 2019 the peak was also in the second half of April when temperatures rose and the pollen season was extended to about 75 days. In comparison, the *Platanus* pollen season in Poznan usually lasted for about three weeks for the period (2005-2009) (Nowak et al., 2012), while the average duration in Granada detected for the period 1992–2019 was 44 days, but with fluctuations ranging from 27 days in 2004 to 74 days in 2017 (Cariñanos et al., 2020).

Similarly, with a maximum in the first half of April, but with a significant increase in temperatures, was the pollen peak during the longest 4-month long pollen season in 2016. In 2017, the peak of *Platanus* pollen emissions was again in the first half of April, but the temperatures were 4.2°C lower than the mean values during the same period of the previous year.

After relatively lower temperatures in 2014, the flowering period in 2015 started later, in the second half of April, and was shorter with more abundant pollen emissions. The flowering period started the latest in 2022, and its peak coincided with the relatively sharp and steady increase in mean hourly air temperatures in Sofia.

The coefficients of variation (CV) for the mean hourly air temperature values found for the month of March ranged between 42% and 105%, with the largest temperature variation recorded in 2013. The variation of temperatures in the month of April was significantly smaller than March peak values, from 28% in 2018 to 66% in 2017, respectively. The stabilization trend in the fluctuations of mean hourly temperatures was also maintained in the month of May, with the CV fluctuating between 19% for the second half of May 2018 and 38% at the beginning of the month in 2019. Temperature fluctuations in June and July in the studied 10-year period ranged between 19% and 28%, respectively, for 2016 and 2017 in the second half of June.

Relative humidity, which is one of the most important indicators that determine the distribution of *Platanus* pollen (Maya-Manzano et al., 2017), in the present study showed greater fluctuations in the period from 2013 to 2017 (Figure 2).

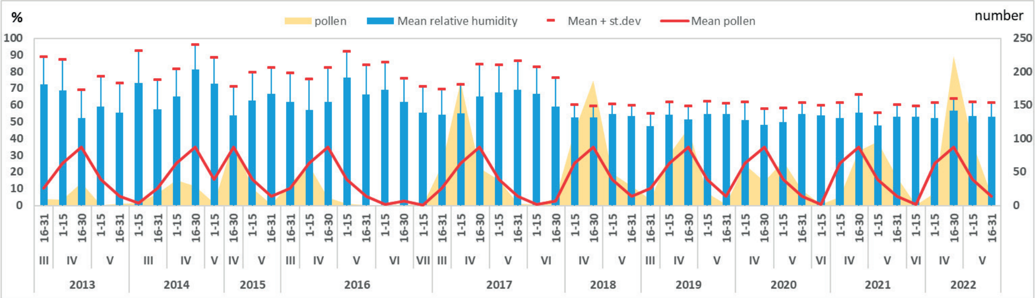

Figure 2. Dynamics of the mean values of the hourly averages of the relative humidity and the amount of pollen during the flowering phase over the period 2013-2022

In most cases, the higher number of pollen grains was associated with a period of lower relative humidity. In the month of March, relative humidity fluctuations varied with a CV between 16% and 31%, and in April between 12% and 33%, respectively. In May, fluctuations above 30% were found only in 2013. For the remaining years from 2014 to 2017, the variation was between 21% and 27%, respectively. For the second 5 years of the studied period, the fluctuations in relative humidity were smaller, between 11% and 17%, respectively. However, the number of identified pollen grains fluctuated widely, between 2 and 95. At the beginning of the studied period, the change in relative humidity in June varied between 23% and 29% while in 2020 and 2021 it was 12%. The atmospheric conditions were also dynamic in June 2016, where the CV of the relative humidity was 28%. A greater number of pollen grains was found at low relative humidity recorded in the period 2013 to 2017, while in the remaining 5-year period the relative humidity fluctuations were weak to insignificant, but the the same correlation was observed again, particularly in 2019 and 2021.

The dynamics of wind speed has a significant influence on the spread of pollen grains, but the observed correlation was ambiguous (Figure 3). In three of the years studied, 2013, 2014 and 2022, respectively, peak pollen amounts were found at relatively lower wind speeds. On the contrary, the highest number of pollen grains was recorded in periods of relatively strong air currents, such as in 2017 and 2020.

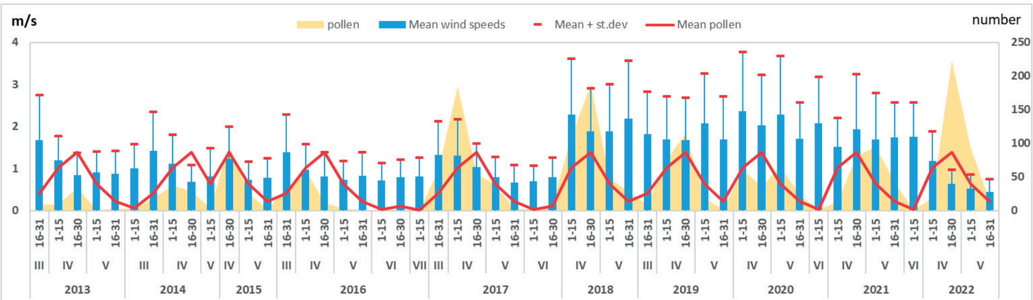

Figure 3. Dynamics of the mean values of the hourly averages of wind speeds and the amount of pollen in the flowering phase over the period 2013-2022

In general, in the period from 2018 to 2021, air currents were characterised by higher speed. At the beginning of the pollen season in March, the variation of the values was large, with a CV between 55 and 66%, respectively. In the second half of April in 2016, fluctuations in the air current speed reached 72%. In the same period of 2021, the wind speed variation was 69%. In the remaining cases, the CV was between 46 and 66%. In the first half of May

2014, wind speed fluctuations were large (CV of 84%), while in May of the other years, the variation was between 48 and 68%. Fluctuations in wind speeds in June and July were between 47 and 58% (Figure 3).

The fluctuations in atmospheric pressure (AP) in Sofia during the studied period were not big (Figure 4).

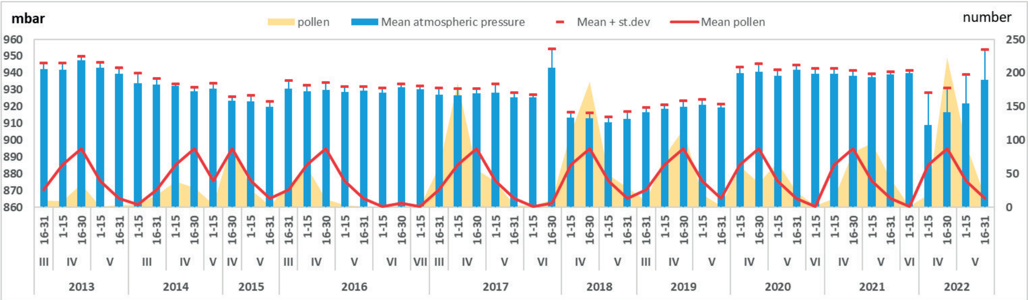

Figure 4. Dynamics of the mean values of the hourly averages of the atmospheric pressure and the amount of pollen in the flowering phase over the period 2013-2022

In March, the values ranged between 0.3 and 0.6%. The dynamics were similar in the following month - April. Only the last year, 2022, was an exception, when the CV was 1.6 and 2.1%, respectively. In May of the same year, the AP varied between 1.9 and 2.0%, but in the other years it was relatively stable with a CV of 0.2 and 0.6%, respectively. The AP was most stable in June and July, ranging between 0.2-0.3%. The only exception was the second half of June 2017, when the AP was higher and the fluctuations were more significant, with a CV of 1.2%. In the said period, the number of pollen grains was 5 times as big as the one obtained for the other years, for which the presence of *Platanus* pollen in the atmosphere of Sofia was reported in June. This could largely be explained with the high AP and the lack of heavy precipitation in that period to clear the atmosphere of pollen grains.

With an increase in solar radiation in spring, *Platanus* flowering intensified. This also occurred in the second half of March in 2014, the first half of April in 2016 and 2017, as well as the second half of April in 2013, 2015, 2018 and 2019 (Figure 5). In 2021, the pollen peak coincided with the increase in solar radiation only in early May, while in 2022, the maximum number of pollen grains was counted in the second half of April, when the solar radiation measured for that particular year was not great. This is largely explained by the higher temperatures and low wind speed during the said period. Slight decreases in the levels of mean solar radiation were found in the first half of April 2019, the second half of the same month in 2014, 2017 and 2022, at the beginning of May 2016, 2018 and 2019 and in the second half of the same month in 2020 and 2021. These decreases can be due to the scattering of solar radiation by particulate atmospheric pollutants, as well as to the absorption of radiation by water vapor in the infrared region of the spectrum, ozone absorption in the ultraviolet region (Tashev et al., 2016).

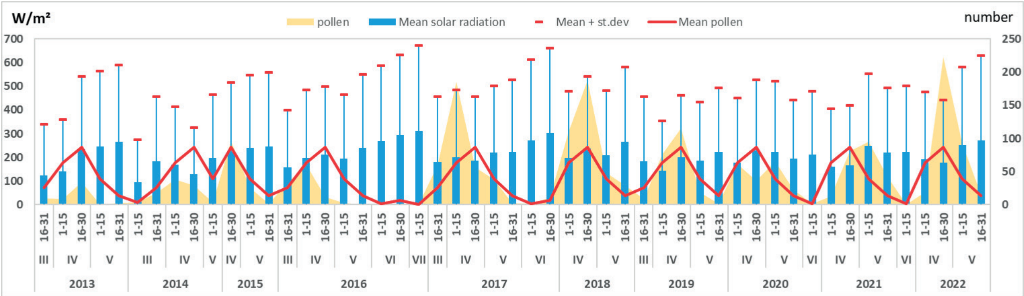

Figure 5. Dynamics of the mean values of the hourly averages of solar radiation and the amount of pollen in the flowering phase over the period 2013-2022

The mean direction of prevailing winds was from WSW (West-Southwest) in March and June to S (South), and in April and May to SSE. The prevailing air currents in the first half of July 2016 were from the SW. Generally, the prevailing winds in 2013 were from S to SW, and in the following 4 years from WSW to S. The prevailing winds in 2021 were from S to SSW. In the first half of May 2018 and 2022, as well as throughout April 2019 and 2020, the recorded winds blew from the SSE direction.

Fluctuations in wind direction are a common phenomenon in the studied area. CV variation in March was between 40% and 55%, in April 32% and 65%, respectively, while in May - 34 to 63%. Fluctuations in wind direction in June and July were also significant, between 37 and 67% respectively. This shows that in the studied period in Sofia Valley, winds the easterly, westerly and even northerly components could often be detected.

Air pollution has been a problem for the city of Sofia for several decades, where the main reason for this is the high concentration of PM_{10} (Doncheva-Boneva et al., 2017). Moreover, *Platanus* pollen may contribute to an increase in PM during the flowering period, consequently aggravating urban air quality (Cariñanos et al., 2020).

The distribution of PM_{10} in the studied *Platanus* flowering period for the last 10 years is shown in Figure 6. The presented maximum mean hourly value was recorded at 1 p.m. on 1 June 2016. It exceeded the previous value by 300 μg/m^3 and was 2.7 times higher than the calculated mean value for the following hour. That fact could hardly be attributed to extreme pollen grain dispersal, even in the immediate vicinity of the Automatic Monitoring Station. During the reporting period, there were other peak concentrations of PM_{10}, which were mainly a result of human activity, not of the pollen season.

The recorded peaks of pollen emissions added to the already polluted atmosphere of the city of Sofia, but their share was relatively small. This trend was more clearly noticeable in 2013, 2014, 2016, 2019, 2020, and 2021. As a whole, in the three years with the maximum number of pollen grains counted (2017, 2018 and 2022) the relative share of pollen in the total amount of particles was greater, as the atmosphere was less polluted with PM_{10}. This showed that with the improvement of air quality in Sofia, the share and importance of tree pollen, and the *Platanus* pollen in particular, will increase.

A significant positive correlation was found between the number of pollen grains and the mean values for the 10-year data series (R = 0.70) on the one hand and wind speed (R = 0.32) on the other. The latter was explained by the fact that stronger wind promoted their further spread, which in turn led to higher pollen levels reported at the aerobiological monitoring station. Conversely, higher values of relative humidity, atmospheric pressure and solar radiation resulted in lower pollen loads. This is also confirmed by the identified negative correlations (Table 1).

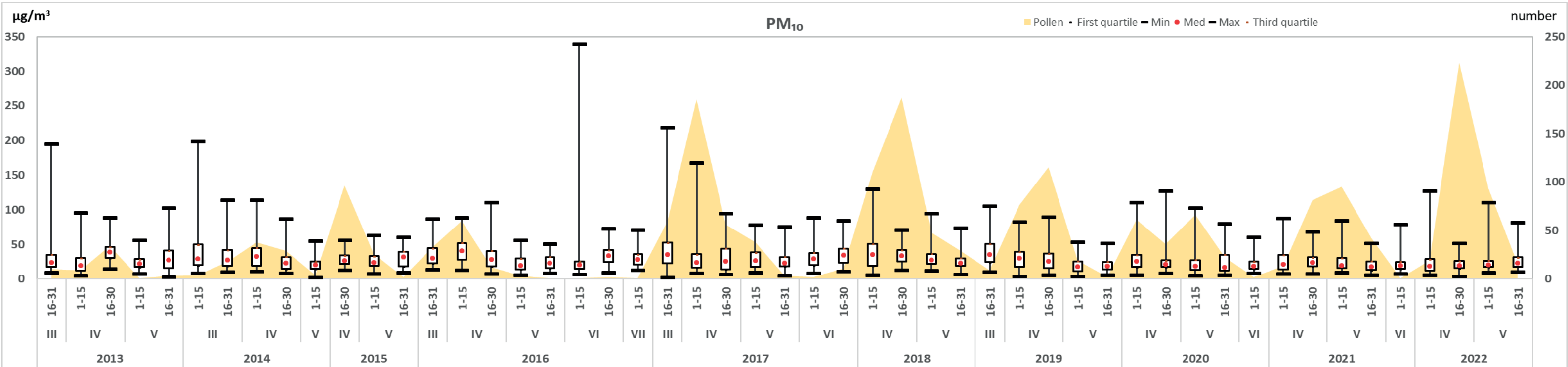

Figure 6. Box-plots of mean values of the hourly averages of PM$_{10}$ contents and the amount of pollen in the flowering phase over the period 2013-2022

Table 1. Spearman Rank Order Correlations (2013 – 2022)

	Mean	AirTem	WS	UMR	Press	GSR	WD	PM$_{10}$
Pollen	**0.70***	-0.26	**0.32***	**-0.45***	**-0.28***	**-0.29***	-0.20	0.20

AirTem – air temperature (°C); WS – wind speed (m/s); UMR – relative humidity (%); Press - atmospheric pressure (mbar),; GSR – general solar radiation (W/m2); WD – wind direction; PM$_{10}$ (µg/m^3)
* correlations are significant at $p < 0,05$

A significant negative correlation between pollen concentrations and relative humidity was reported (Sánchez-Reyes et al., 2009; Álvarez-López et al., 2022).

Rosianu et al. (2022) found a positive correlation between the concentration of pollen and particulate matter PM_{10}.

CONCLUSIONS

The results of the study showed that the meteorological factors which directly affect the concentrations of airborne pollen were wind speed, relative humidity, atmospheric pressure and solar radiation.

For the time being, the total amount of *Platanus* pollen in Sofia is relatively low. However, in recent years there has been an upward trend. That growth trend is expected to continue due to the degree of maturity of *Platanus* specimens. The use of that species as a dominant element in the urban green infrastructure of Sofia should be reconsidered, not only to improve air quality, but also to enhance urban biodiversity, its resilience and ecosystem services.

In future research, a continuous aerobiological monitoring and a long term and large-scale clinical study on the allergenic potential of each *Platanus* species should be conducted. Designing a *Platanus* pollen calendar and making it publicly available would be a preventive public health measure.

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IMPACT OF PERENNIAL FLOWER PLANTS USED IN LANDSCAPES IN BUCHAREST

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Abstract

Urban landscaping such as green squares, flowerbeds and green pills are the most common, most numerous and most exposed to traffic. Cities in the European community and major cities worldwide are increasingly using perennial flowering plants in urban landscaping and investing in research into the adaptability of these types of plants to harsh urban conditions. Bucharest is only at the beginning of introducing perennial flowering plants in the design of these types of green spaces, as there are no studies on species that are resistant to the level of pollution and environmental conditions of this city. This paper discusses the impact of perennial flowering plants in urban landscaping in Bucharest, in heavily trafficked road and pedestrian areas, presenting both the problems encountered and the good things about the landscaping choices made by the municipality. At the same time, the paper aims to demonstrate the need for the involvement of the municipality and the specialist community in carrying out comprehensive studies on the resilience of perennial flowering plants in the current urban space of Bucharest, as on their use.

Key words*: Bucharest, perennial flower plants, urban green spaces, urban landscape.*

INTRODUCTION

"Landscaping a green space is not about filling the empty spaces between buildings with stones, grass, flowers and trees, but about organizing, harmonizing and disciplining plant forms, the dynamic chromatics of flowers and leaves, the mobility of water, the textural contrast of surfaces and relief with different buildings or amenities."

For each individual, depending on the type of viewer (resident or tourist, intellectual or worker, old or young, parent or child), the perception and experience of the urban space in which they live is different. Contemporary civilization is basically defined by the evolution, dynamics and development of cities. Important elements of urban space include landscaping. Of these landscapes, squares, flowerbeds and green pills are the most common, the most numerous and the most exposed to road and pedestrian traffic and high thermal radiation in summer. Due to the polluted environment (air and soil), these types of landscaping have a greater and more sensitive degree of difficulty in their design, raising problems in maintenance work as well. In these types of urban spaces, from an aesthetic point of view, flowers contribute to satisfying people's need for beauty, as their colors have a beneficial psychological influence on us.

The paper presents a visual analysis of the impact of perennial flowering plants used in the above-mentioned types of urban development in Bucharest.

The study is important in order to establish a support (technical, aesthetic and ecological) for this type of landscaping.

It should be noted that there is currently no study on perennial flowering plants in this respect (studies of resistance to environmental factors, principles of arrangement and maintenance, etc.) in the urban environment of Bucharest. Thus, the urban landscaping with perennial flowering plants that I will be analyzing has been carried out over the last 10 years by the local authorities of Bucharest without any specialized studies.

MATERIALS AND METHODS

1) University Square (Piața Universității) is a very important area of the capital from several points of view (urban, architectural, cultural, historical, social, tourist, etc.) located right in the center of the capital, at the intersection of Regina Elisabeta, Nicolae Bălcescu, Carol I and Ion C. Brătianu boulevards, creating the north-south and east-west axes. The shape of the intersection is of Parisian influence after the Haussmannian model of the "Grand-Intersection" (La Grande Croisee). At the same time, this intersection is home to several buildings of great architectural value of important institutions such as the University of Bucharest, the hotel Grand Hotel Bucharest (former Intercontinental), National Theatre "I. L. Caragiale" Bucharest, the Ministry of Agriculture and Rural Development, Colțea Clinical Hospital and the Museum of Bucharest. It is an intersection located in the Protected Area no. 4 - Magheru - Brătianu, with very intense traffic both by road and by pedestrian and represents one of the main emblems of the capital, thus becoming a much-visited area by tourists. In this context, the aesthetic function of the layout of the squares formed at this intersection is of high importance (Figure 1).

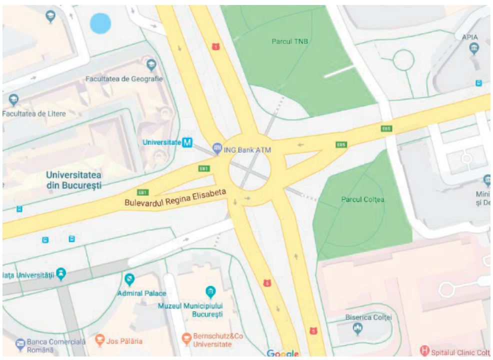

Figure 1. University Square, map - retrieved from Google Maps (image source: https://www.google.ro/maps/@44.4357114,26.1027727,18.5z?hl=ro)

On these squares the municipality has realized in 2013-2014 round groups formed by: *Yucca filamentosa, Juniperus horizontalis* interspersed with bulbs of *Tulipa* sp., *Rosa ground cover* interspersed with bulbs of *Tulipa* sp., *Miscanthus sinensis, Kniphofia uvaria* withouter circle formed by *Aquilegia vulgaris*, round in which they have placed centrally a species of *Catalpa bignonioides* Nana. These circles are linked by groups of plants, without a clear geometric shape but sinuous, consisting of *Berberis thumbergii* interspersed with bulbs of *Tulipa* sp. and *Leucanthemum vulgare* interspersed with *Berberis thumbergii* and surrounded by turf (Figures 2-6).

Figure 2. University Square, view towards the central square (date image taken: 27.06.2017)

Figure 3. University Square, view towards Queen Elisabeth Boulevard (image capture date: 27.06.2017)

Figure 4. University Square, view towards Ion C. Brătianu Boulevard (date image taken: 27.06.2017)

Figure 5. University Square, view from the square on Ion C. Brătianu Boulevard (date image taken: 27.06.2017)

Figure 6. University Square, overlooking the National Theatre "I. L. Caragiale" Bucharest (date image taken: 27.06.2017)

All areas are sprinkler irrigated.

2) Charles de Gaulle Square is another important intersection in the capital, linking the northern exit of the city to the main boulevards leading to the city center (Figure 7) and is part of the Protected Area 14 - Aviators.

Figure 7. Charles de Gaulle Square, map - retrieved from Google Maps (image source: https://www.google.ro/maps/@44.4658748,26.0866541,18z?hl=ro)

This intersection "underwent" a big change of image with the redevelopment of the intersection in 2015 by the local authorities, and the creation of a more vertical concept in terms of image than the previous one and more abundant in terms of the multitude of species planted. Basically, the squares formed in this intersection, were landscaped with massive and dense groups of trees (*Salix* sp.) with various species of shrubs and perennial flowering plants such as *Rudbeckia fulgida*, *Iris* sp., *Lavandula* sp., *Coreopsis* sp., *Achillea millefolium,* various *decorative grasses*, etc. (Figures 8-12).

Figure 8. Charles de Gaulle Square, view from the square on Aviatorilor Boulevard (date image taken: 23.07.2017)

Figure 9. Charles de Gaulle Square, view from the square on Aviatorilor Boulevard (date image taken: 23.07.2017)

Figure 10. Charles de Gaulle Square, view towards the central square from Mareșal Constantin Prezan Boulevard (date image taken: 23.07.2017)

Figure 11. Charles de Gaulle Square, view towards the central square from Aviatorilor Boulevard - entrance area (date image taken: 23.07.2017)

Figure 12. Charles de Gaulle Square, view towards the square in the area of Spring Avenue (date image taken: 23.07.2017)

The irrigation system is combined: sprinkler irrigation for lawn areas and surface drip irrigation for areas with groups of plants.

3) Unirii Boulevard (Bulevardul Unirii) - the section between Unirii Square and Alba Iulia Square (Figure 13), is a wide artery with green spaces such as green flatbeds running along the entire length of the boulevard both on its sides and on the central axis.

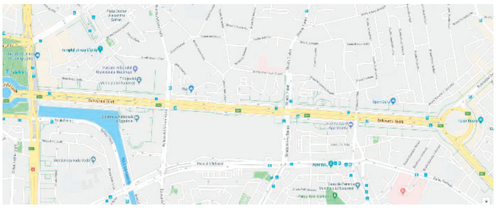

Figure 13. Unirii Boulevard, map - retrieved from Google Maps (image source: https://www.google.ro/maps/@44.425593,26.1167538,15.58z?hl=ro)

The existing vegetation on the central plateau was enriched, in 2014, by planting species of roses that bloom throughout the warm season (Figure 14). The end of this plateau facing Alba Iulia Square was landscaped with annual floral "spots" (*Begonia semperflorens* Rouge/Red) (Figure 16).

The sidewalks (two on each side) were altered in terms of terrain by creating a slope with a slope running down to the street for the street ones, which were also planted with clusters of roses. In areas with intersections, and interior promenade areas, the flatbeds were transformed into large planters by building enclosed retaining walls around them and raising the ground level by 40-50 cm. Among the existing trees, plant clusters were created consisting of dwarf shrubs decorative by foliage such as *Euonymus fortunei, Berberis thunbergii Atropurpurea* and *Juniperus horizontalis* and perennial flowering plants such as *Lavandula* sp., also a decorative species and by foliage (Figures 14-15).

Figure 14. Unirii Boulevard, view towards the central and side street (date image taken: 30.09.2016)

Figure 15. Unirii Boulevard, view to the sidewalk promenade (date image taken: 30.09.2016)

Figure 16. Unirii Boulevard, view towards the central platform - end of Alba Iulia Square area (date image taken: 30.09.2016)

At this location, the irrigation system is also combined: sprinkler irrigation for the lawn areas and surface drip irrigation for the cluster areas.

RESULTS AND DISCUSSIONS

1) By analyzing the first area, **University Square**, we discover an urban development with many problems from many points of view. For 70% of passers-by (according to the survey conducted on a sample of 100 people divided into four age categories and categories of pedestrians and drivers), from an aesthetic point of view, the landscaping tends to go unnoticed and indifferent to the viewer's eyes. It does not attract attention, is pleasing to an average level and does not disturb the eye.

Compositionally it is a rather monotonous arrangement with few accents due to the flowering periods, which are short and unbalanced.

Due to inadequate maintenance of these species (such as: sprinkler irrigation and inadequate water quantity, lack of regular fertilization, lack of species-specific and regular maintenance work - pruning, dry cleaning, weeding, etc.), the plants have developmental problems (Figures 17-21). Because of these developmental problems, a correct assessment of these perennials in terms of resistance to environmental and pollution factors is compromised. Thus, the rondo formed by *Kniphofia uvaria* with an outer circle of *Aquilegia vulgaris*, has completely dried out, and since 2019 annual flowers have been planted on its surface (Figure 22). At the same time, the free areas remaining after the drying of some *Yucca filamentosa* specimens, as well as some shrubs in the arrangement were filled with annual flowering plants (Figures 23-24).

Figure 17. University Square, *Kniphofia uvaria* with outer circle of *Aquilegia vulgaris* (date image taken: 27.06.2017)

Figure 18. University Square, *Leucanthemum vulgare* (date image taken: 27.06.2017)

Figure 19. University Square, *Yucca filamentosa* - water regime problems (date image taken: 27.06.2017)

Figure 20. University Square, *Rosa ground cover* interspersed/strewn with bulbs of *Tulipa* sp. (date taken image: 27.06.2017)

Figure 21. University Square, *Berberis thumbergii* interspersed/interspersed with bulbs of *Tulipa* sp. (date taken image: 27.06.2017)

Figure 22. University Square, former round of *Kniphofia uvaria* and *Aquilegia vulgaris* - replanted with annual flowers (date image taken: 09.2022)

Figure 23. University Square, round with *Yucca filamentosa* - completed with annual flowers (date image taken: 08.2019)

Figure 24. University Square, areas with shrubs - completed with annual flowers (date taken picture: 10.2022)

The only species less affected by poor maintenance, and which has shown good resistance over time to urban pollution, is *Miscanthus sinensis*, which has been growing well over the years, with a pleasant and decorative appearance, only in the last 2 years some specimens have dried out (Figures 22 and 25).

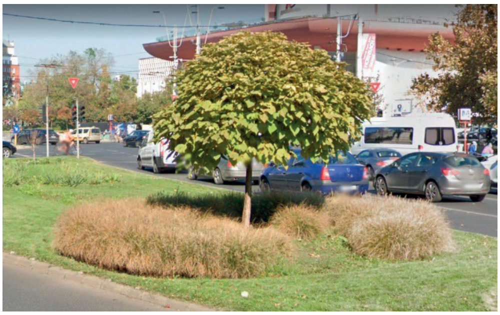

Figure 25. University Square, round decompleted with *Miscanthus sinensis* (date image taken: 10.2021)

In the second half of 2019, the entire landscaping of the central square of the intersection, including the areas with

Miscanthus sinensis, was dismantled for the construction of the Ion Constantin Brătianu monument. This aspect is of importance in the analysis of the evolution and development of *Miscanthus sinensis* specimens, because by shrinking the sample the analysis and conclusions were affected.
From the pavement, at a distance of at least 10m from the analyzed squares, practically from the pedestrian viewer's point of view, these plant problems cannot be observed. Visually, it is just an overview that can be more colorful or bland depending on the season, with areas with small gaps depending on the specimens dried and removed from the group.

2) Compared to University Square, the landscaping of **Charles de Gaulle Square** creates an extremely strong visual impact on passers-by, but not in a good way.
For 85% of passers-by in this area (according to a survey of 100 people divided into four age groups and categories of pedestrians and drivers), from an aesthetic point of view, the landscaping creates a "wild" image, influencing passers-by's perception of the space and creating a general feeling of discomfort. The majority of interviewees stated that they felt as if they were in the "delta".
Drivers, especially those who do not live in Bucharest and those who have to cross this intersection for the first time or very rarely and do not know the area well, can no longer visually navigate the intersection due to the tall and abundant vegetation - creating a feeling of disorientation in space.
The only species of perennial flowering plants that can be seen and admired by passers-by are the few specimens planted in the small, triangular squares formed at pedestrian crossings. Several hundred more perennial flowering plants have been planted in the plant clusters created. These, due to the planting position and the mixtures made with the shrub species, were almost visually obliterated. The only species that managed to stand out and provide a splash of accent and color to the arrangement was *Rudbeckia fulgida*.
In general, the chosen species have a good resistance to urban environmental factors. But the choice of more water-loving species, combined with species that prefer a different water regime, and their planting in an intersection, which in the hot season turns into a real "radiation furnace", has created various types of development problems for the planted species. Some of these problems are: harbor depreciation, suffocation, drying out of some specimens until the disappearance of some species from the arrangement. Among the most affected perennial flower species are *Iris* sp., *Achillea* sp., *Coreopsis* sp., *Rudbeckia* sp. (Figure 26), and among the most resistant species are decorative grasses (Figure 27) and *Lavandula* sp. (Figure 28).

Figure 26. Charles de Gaulle Square, *Coreopsis* sp. (date taken image: 23.07.2017)

Figure 27. Charles de Gaulle Square, decorative grasses. (date taken picture: 09.2022)

Figure 28. Charles de Gaulle Square, *Lavandula* sp. (date taken picture: 09.2022)

3) The improvement of **Unirii Boulevard** (the section between Unirii Square and Alba Iulia Square), carried out in 2014, has a positive impact on passers-by.
For 75% of passers-by in this area (according to a survey of 100 people divided into four age groups and categories of pedestrians and drivers), from an aesthetic point of view, the landscaping creates a pleasant and modern image.
The composition of the plant groups is balanced. Each species is visible, harmonizing with adjacent species and highlighting each other through the color of their habit.
As well as roses, passers-by were delighted to recognize lavender plants.
The whole arrangement is well maintained, with all pruning and cleaning work carried out at the required times, always giving a well-groomed appearance.
From a driver's perspective, the landscaping creates a pleasant environment and there are no elements of it to disturb the smooth flow of traffic.
A negative and important mention of this development is that by elevating the land, existing mature trees have been affected. The newly added topsoil has surrounded their stem to a height of 40-50cm. This layer prevents the roots of the trees from breathing and maintains continuous moisture directly on the bark, which in time leads to rotting and facilitates the attack of diseases and pests on the trees. In practice, this over-elevation of the soil affects the biology of the trees, the effects of which can be observed over time (Figure 29).

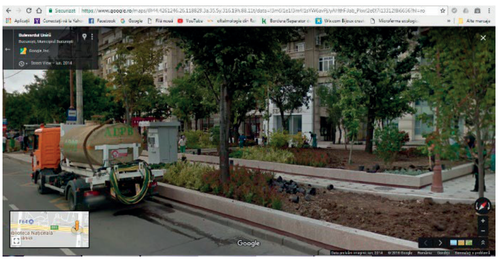

Figure 29. Unirii Boulevard, elevation of the sidewalks - Google Maps (image source: https://www.google.ro/maps/@44.4261246,26.118829,3a,75y,325.48h,99.22t/data=!3m6!1e1!3m4!1sYW6avPjJyAHthFiJab_Pkw!2e0!7i13312!8i6656?hl=ro)

The municipality has chosen to use only one species of perennial flowering plant (*Lavandula* sp.) in the landscaping solution. The large surface area of these street beds, which are approximately 2 km long, offers the opportunity to landscape them with a much wider range of perennial flowering plant species. By using these, the plant base would be enriched and ecological and aesthetic diversity would be created.

CONCLUSIONS

These three areas analyzed are among the first major urban landscaping projects in Bucharest, where the municipality used perennial flowering plants in their landscaping.
The dates (years) of the creation of these landscapes are important in analyzing the resistance of the perennial flower species used to urban environmental factors.
Visual analysis over time has shown that the main factors contributing to the degradation of the landscapes are the way they are laid out, the species association and inadequate maintenance.
Given the number and size of urban green spaces throughout the capital, the use of these types of plants would require the municipality to invest in sustainable landscaping, whereby the maintenance costs would be much lower than the maintenance needs of lawns and annual flowering plants. In addition, the use of perennial flowering plants in the landscaping of urban green spaces in Bucharest means enriching the plant base and its biodiversity, aspects that contribute to improving environmental factors and quality of life.
In order to achieve such landscaping with perennial flowering plants in an efficient, economical and aesthetically balanced/ harmonized way, it is necessary that both the municipality and the specialist community engage in comprehensive studies on the resilience of perennial flowering plants in the current urban space of Bucharest and on their use. These studies should result in a "regulation"/"catalog" that provides for perennial flowering plant species resistant to the urban environments and pollution factors of Bucharest. At the same time, the species should be organized by ecological needs (light, water

regime), flowering periods, colors, height regime, foliage type, bush diameters, and any other necessary elements identified. Such a system of organization of perennials can contribute positively to the choice of appropriate species in the landscaping of urban green spaces in Bucharest.

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INTRODUCING ARCHITECTURAL-LANDSCAPE ELEMENTS IN URBAN SQUARES FOR HIGHER QUALITY OF LIFE

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Abstract

The value and attractiveness of landscaped green spaces stem from their ability to satisfy the general need for urban comfort and quality of life. This study suggests a redevelopment of Independence square by introducing architectural and landscape elements ensuring a higher degree of attractiveness. The square is located in the center of Iasi between Independence Boulevard and Vasile Conta Street, creating a large urban triangle with Union and Mihai Eminescu Squares. The redevelopment solution was created and presented in detail considering site features, using practical functional and urban composition research. The aim of this proposal was to meet such current goals as creating a comfortable urban environment for relaxation, socialization, and contemplation but also raising the quality of daily life and free-time spending in a pollution-free environment. The suggested concept tried to create a unitary and multifunctional space covered by a set of landscaping effects that harmoniously integrate the area into the urban tissue of Iasi.

Key words*: landscaped green areas, redevelopment solution, urban environment, urban tissue*

INTRODUCTION

As population density in cities increases, the quality and sustainability of urban living environments become increasingly important (Kozamernik. J et al., 2020). Urban green spaces play a vital role in enhancing urban biodiversity and sustainability, contributing directly to human health (Wu et al., 2021). In a heavily anthropized environment, green areas have become essential due to their beneficial effects on the environment through lower pollution, as well as on citizens by providing higher urban comfort and quality of life. Lately, urban green spaces have been increasingly attributed the quality of mitigating the negative psychophysiological effects that people suffer from in densely built environments (Velarde et al., 2007). Moreover, several studies have concentrated on the expansion of green areas on roofs (Zlati et al., 2022) and building facades (Cojocariu et al., 2022a; Cojocariu et al., 2022b) in order to increase the share of green areas in urban environments,

Green squares reflect a unity of green space in an urban environment, which plays a key role in city development. These urban green spaces with a valuable aesthetic component (Kaplan et al., 2006) contribute to improving the quality of life by providing to city inhabitants relatively inexpensive opportunities to connect with nature in everyday life (Wang et al., 2019). Their accessibility and openness often turn them into centres of urban events and social nodes. Squares are green spatial cut-outs providing a breathing area in the middle of constructions, a lung in the urban context (Haq, 2001), an ideal space for socialization and human interaction. They can provide an environment conductive to the development of social life leading to a stronger sense of community and therefore ensuring a higher level of social interaction. So, there are multiple advantages brought by these landscaped green spaces leading to physical, psychological, aesthetic, and social benefits. Therefore, a longer human contact with nature contributes to physical and mental health (Nordh & Østby, 2013) and a longer life.

Vegetation, furniture, and water are the main elements of a landscaped green space, which, organized in a harmonious and coherent way could generate varied impressions, starting from freedom and naturalness to artificiality and rigidity, from melancholy to joy. All these morphological elements work together in landscape compositions through shapes, colours, textures, and volumes, the manner in which they are linked together enhancing their effect. Vegetation, especially through colour and texture of used plants directly influences mood, bringing to surface memories and emotions. Using plant elements with various textures is a great way to create diversity in a landscaped green space. Colour is highly important in landscape design, adding dimension and interest to any development (Cantor et al., 2018).

Seating places are apparently trivial objects but indispensable in urban green spaces. They should provide great comfort and also be closely linked to the green space. To ensure a high quality of urban space and a pleasant experience, urban furniture items should have several main properties, such as comfort, security, shelter, and design. (Mexi & Tudora, 2012).

Due to its aesthetic and therapeutic qualities, water completes the other morphological landscape elements (Faggi et al., 2013). Being a visually attractive element, water brings charm to a landscape, managing to enhance its value. In turn, water should be enhanced by ingenious landscaping, balanced architectural elements, and also by subtle plant arrangements (Dascălu & Cojocariu, 2016).

MATERIALS AND METHODS

Independence Square is located in the centre of Iasi between Independence Boulevard and Vasile Conta Street, creating a large urban triangle with Union and Mihai Eminescu Squares (Figure 1). The historical Independence Statue is located here, a symbolic element of the square bearing the same name. The Myth of the Mothers Church, the Saint Spyridon Church, Doctor Ludwig Russ Monument, and the Craftsmen's Insurance House are the other historical monuments in the immediate vicinity of the square.

The Independence square appeared due to city reorganising. After the 1977 earthquake, Independence Boulevard was extended and rearranged, being renovated as a main street in 1980. The Independence Statue was made by Gabriela Manole-Adoc and Gheorghe Adoc after a national competition in 1975, aimed to celebrate the centenary since the proclamation of national Independence in 1877. After the passing of time, the pedestrian alleys and access ways to Independence Square reached a state of disrepair, the steps to the historical monument being damaged and detached and becoming a danger for the pedestrians in the area.

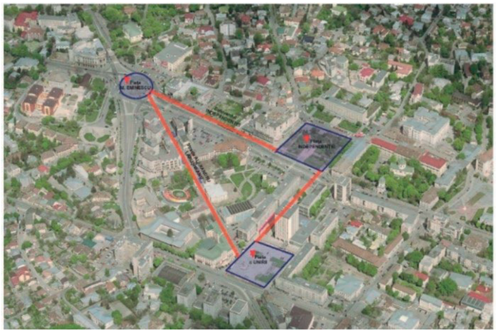

Figure 1. Urban triangle: Independence-Union-Mihai Eminescu Squares Aerial view

Therefore, square redevelopment was highly necessary. The statue plinth and the pedestrian walkways were repaired in 2019. Plus, a part of the old undecorated plant elements was removed and new plants were added, being mainly grouped in oval-shaped areas of the square.

Today, Independence Square is partially exploited in its urban context. This study aims to provide a square redevelopment landscaping to shape a new perspective to the area and enhance the landscaping value of the site. The main goal is to animate the green space in the urban context and put an accent on the landscaping effects of the newly introduced morphological elements. Although this area has been recently rehabilitated, it lacks strategically placed natural and mineral elements so that the place could provide new experiences to the strollers.

RESULTS AND DISCUSSIONS

Public or semi-public spaces are the places maintaining and feeding a human need of interaction, exchange of ideas, and relaxation, while also providing a place where events take place. The main feature of such a space is provided by the functionality and aesthetics of the morphological elements of the landscape

design, which are placed in public spaces and where the main focus is being placed on the effects of such elements on users. There are spaces not reaching their full potential in every city due to a wrong approach or unsustainable exploitation (Pașcu et al., 2021).
However, the application of principles aimed at building harmony and unity in diversity may lead to a composition with an attractive and sustainable architectural silhouette (McPherson & Peper, 2012).
Yearly, hundreds of new hectares of green spaces are designed and arranged - gardens, parks, squares, plantations along traffic arteries, green spaces next to residential areas, along tourist routes, which, irrespective of their style, provide landscapes with multiple functions, such as work and rest stimulation in a pleasant and healthy environment.
Green space arrangement is a long process that involves successive steps of putting into practice the thinking behind the design created in an office. The exploitation of this potential and obtaining the effects of high aesthetic value depend not only on a well-thought distribution and balanced combination of the elements but also on their care as the preservation of such elements over time relies on it.

Description of current state

The Independence Square with a surface of around 6800 sqm has an almost rectangular shape in plan.

Figure 2. Aerial view of Square (Google Earth)

There is access to the market from all its sides through perimeter pedestrian circulation. Besides these, the square is bordered by traffic lanes on three sides (to the North, South, and East). To the west, the square is connected by a series of planted spaces and tiered planters with the adjacent built complex aligned to the Independence Boulevard (Figure 2).
The existent formal concept is mainly based on orthogonal shapes with small angular interventions or curves in the area behind the statue towards Vasile Conta Street.
The studied location is both a pedestrian circulation node, a central and cultural area, a symbolic place due to Independence statue placement, as well as a place of social expression for important political events. All these coexisting dimensions should be put on a single plan by creating a suitably landscaped space.
Due to its central location in the city in the middle of historical and public utility buildings, the main function of Independence Square is that of transition and partially relaxation.

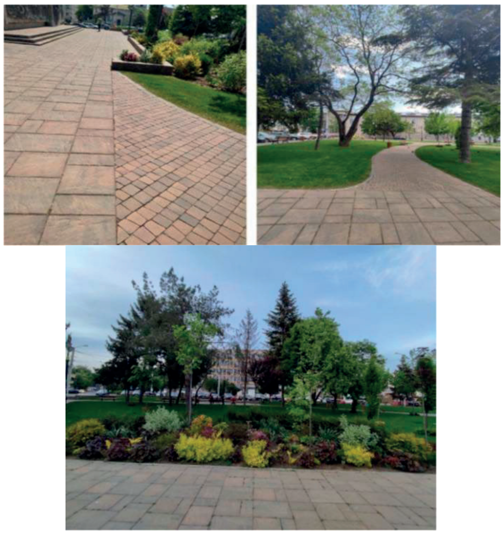

Figure 3. Current state – Pictures *(original)*

In terms of vegetation, there is a varied assortment of used trees and shrubs (Figure 3). Specimens of trees from the species of *Abies concolor*, *Aesculus hippocastanum*, *Acer platanoides*, *Acer negundo*, *Betula pendula*, *Carpinus betulus*, *Catalpa bignonioides* 'Nana', *Morus alba*, *Picea abies*, *Pinus strobus*, *Platanus occidentalis*, *Ulmus pumila*, *Tilia tomentosa* are randomly found in the square perimeter. Such shrubs as *Berberis thunbergii*, *Cornus alba*, *Juniperus horizontalis*, *Weigela florida, Euonymus fortunei* are planted together in plant compositions alongside such flowering species as *Canna indica*, *Lavandula angustifolia*, *Heuchera* sp., *Hosta* sp., *Yucca filamentosa*, and ornamental grasses from the *Festuca*, *Stipa,* and *Miscanthus* species.

Description of constructive solutions and analysis of suggested functional areas

The entire suggested square ensemble is dominated by a geometric style and is based on regular geometric shapes, made of straight rectangular lines. General effects and appearance of the arranged square suggest order and formality, also strongly conveying tranquillity and contemplation through the finesse of landscaping arrangements.

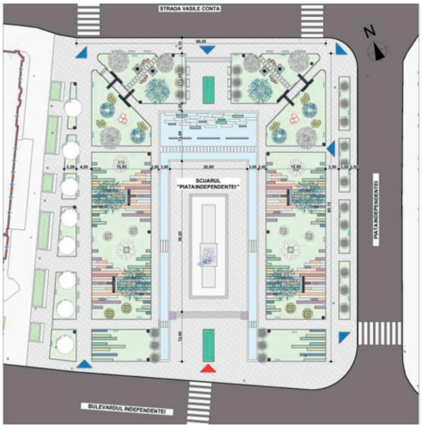

Figure 4. Arrangement plan

Although justified by the existent anthropized framework comprising buildings with a strong representative character, the organic makes its presence felt by means of plant elements. The plants are arranged orderly and geometrically, while plant material seems to be subordinated to the built elements. This creates an effect of hierarchical submission denoting order and rigour.

The overall composition was generated by the rectangular geometry of the site defined by linear alleys dividing the entire development into four landscape areas (main access, central interest, relaxation, promenade), each having its own identity and being harmoniously connected to each other (Figure 4).

The central axis expressed by the Independence Square, for which, a dual composition approach was preferred for the pedestrian alleys accompanying the median axis on both sides, being also supported by the water mirror is an interesting particularity of the square, which is highly exploited in the landscaping. This solution gives a dominant force position to the mineral defined by the statue underlining further the main theme and purpose of the Independence Square.

The main access area serves as a meeting place and introduction to the space to be explored and travelled along the central axis. The central area is also the centre of gravity of the composition, where the Independence Statue is located - the main attraction point of the square. The promenade area invites the public for a walk and transitions through the square decorated with pergolas and resting places, the resting area containing relaxation and recreation spaces.

To create a floating effect of the statue, a mirror of water around it is suggested. This way, the value of statue appears much better. Also, it is suggested that pergolas covered in decorative vegetation should be put in place to increase the attractiveness of the square and extend the amount of time spent by the inhabitants here. Their purpose is to provide shadow areas for the space that would bring a new aesthetic and functional perspective.

Each landscaped area was based on the idea of meeting the needs of the general public and be accessible to all ages, starting with children who can play, explore and feel the water underfoot in the water mirror arranged on the central axis, and ending with teenagers and adults who can enjoy nature in a harmoniously arranged environment designed for relaxation. It has also been suggested for some routes to be restored.

General arrangement of square composition was done considering the central area of interest defined by the Independence Statue, the main element accompanying the ensemble along the long line towards Vasile Conta Street. Here the water mirror plays a key role in creating the effect of tranquillity that could be quickly contrasted by its dynamism using the water jets suggested on the central axis of the composition (Figure 5).

Figure 5. Water play

Figure 6. Aerial view of the square area

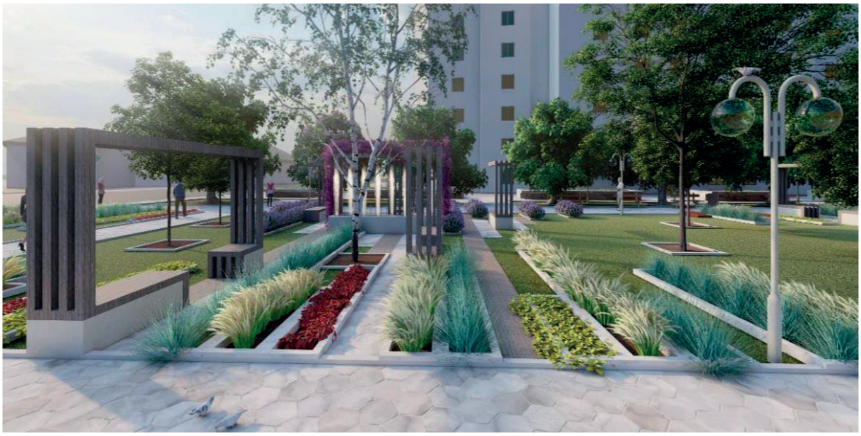

Figure 7. Vegetation square

The studied land plot is a relatively flat requiring a more dynamic approach for avoiding the monotony of the site. To achieve a more animated setting (Figures 6 and 7), there were used colours were used (purple from flower species, green from trees and ornamental grasses, cherry from shrubs); contrasting textures of warm laminated wood and cold and hard stone, mineral elements (furniture arranged in a line in the relaxation areas); natural elements (various vegetation decorating by means of leaves, flowers, bark), being followed a contrast between the natural and the built (the heavy and massive statue floating on the water mirror).

Water is an essential element used in the rearrangement and revitalization of the square. Water washes, purifies, and signifies meditation and the return to the pure spirit within us. The presence of a water surface has become necessary in the arid and polluting urban environment. Several studies have shown that people see as more attractive a green space containing this element (Faggi et al., 2013). Water, like a transparent mirror, generously offers its naturalness and creates a state of tranquillity. Therefore, as it has been mentioned above, we decided to have a fine line of water on the perimeter of the statue as to underline the fragility of water in contrast with the hardness of stone and grandeur of the monument. To animate the area overseeing Vasile Conta Street, the suggestion was made to use water jets as a natural element, whose dynamics helps in generating ozone around water.

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The alleys are another element playing an important compositional role in square development and in the creation of high-impact landscape effects for users. They directly focus on the geometric character generated logically by the leading lines to attraction and recreation points. The relaxation space is highlighted with the help of seating places made of rectangular laminated wood, metal and concrete elements arranged linearly (Figure 8) on the access path, which stem from the horizontal pavement, climbing continuously until turning into pergolas. The pergolas are covered with the plants belonging to *Wisteria sinensis* species providing shade for the seating areas on hot summer days, as well as comfort and coolness to the public. The promenade area invites the visitor to discover gradually lianas decorated pergola arrangements, while the seating place accessible under the pergola reveals where an amazing scenery where *Yucca filamentosa* is the main element of the image projected on the vertical elements of the pergola (Figures 9 and 10). The contrast is accentuated by the green colour in the vegetation reflected on the bright white colour of the marble. In the sub-zones inside the park, there could be noted a subtle transition from the force of the geometric composition to the free-organic style achieved using the arrangement of flower species, shrubs, trees and ornamental grasses. After the analysis of site vegetation, it was suggested to keep a part of what there is now. The proposed plant compositions were arranged following the principles of composition - scale, proportion, rhythm, harmony, balance. They were arranged at different heights, and aesthetically harmonized with the landscape through colours, rhythms of shapes and volumes that will be gradually decorating each season, including winter.

There were suggested the following landscaping species of trees: *Betula pendula, Acer platanoides, Acer negundo*, *Catalpa bignonioides* 'Nana', *Tilia tomentosa*, *Picea punges* 'Glauca' and shrubs *Berberis thunbergii* 'Crimson pygmy', *Cornus alba* 'Sibirica variegata'. To complete the selection of plants for green square arrangement, we have also used such ornamental grasses as *Festuca glauca*, *Stipa tenuissima* 'Pony tails', *Miscanthus sinensis* 'Gracilliamus', as well as flower species *Lavandula angustifolia*, *Heuchera vilosa* 'Palace Purple' and *Hosta undulata* 'Mediovariegata'.

Figure 8. View from the east side

Figure 9. The promenade area arranged with pergolas

Figure 10. View from the southeast side

CONCLUSIONS

Green spaces are spatial cut-outs in the context of urban settlements with a key role for better environment. Vegetation, a living component of nature, regulates the cycle of oxygen and carbon dioxide, enriches the atmosphere with oxygen, and reduces carbon dioxide in the atmosphere. Nature provides an unlimited source of textures, shapes, colours – the elements that have been subtly exploited in the arrangement of the square in order to create harmonious and contrasting effects between the built and natural components. The presence of water in the Independence square arrangement is a point accentuating the central area around the statue and shaping a relaxing and peaceful atmosphere in line with the goal of this redevelopment. Water is also used to stimulate the senses. So, visitors have the opportunity to explore and feel how cold water flows under their feet on a summer day by touching the stones placed on the water mirror. Apart from these advantages, the public could benefit from the value brought by the natural elements meeting the need for recreation and relaxation in a comforting environment.

The solution for Independence square arrangement was created and presented in detail in line with location specificity and derives from functional and urban composition research. The aim of this proposal was to meet such current goals as creating a comfortable urban environment for relaxation, socialization, and contemplation but also raising the quality of daily life and free-time spending in a pollution-free environment. The suggested concept tried to create a unitary and multifunctional space covered by a set of landscaping effects that harmoniously integrate the area into the urban tissue of Iasi.

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RESEARCH ON THE BEHAVIOR OF ORNAMENTAL SPECIES IN SALINE SOILS CONDITIONS

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Abstract

Although over the years, the cut flower industry has faced various challenges, it still remains an important sector of agriculture. Globally, in recent years, the production of cut flowers has increased. This can be associated on the one hand with the low impact on the environment, but also with their ecological effect. Apart from the traditional cut flowers that require large inputs of energy, there can be other ornamental species that can successfully fulfil multiple roles: improving ecosystem services as well-being through colour and attracting insects, economically easy to maintain and last but not least, bioremediation and implicitly, the valorisation of degraded land by natural or anthropogenic factors. Thus, the paper aims to present the behaviour of some ornamental species cultivated in different concentrations of salinity, as follows: S1 - 4,310, S2 - 12,330, S3 - 8,050, S4 - 5,760, S5 -18,630, S6 -24,600 mS/cm. As ornamental plant species were used Limonium sp., Celosia sp., Gypsophila sp., Amaranthus sp. During the experiment, seed germination and biometric measurements as plant height and number of leaves of the plant species were monitored. Also, before and after the experiment, for each variant of cultivation substrates, pH and electrical conductivity (EC) were registered. According to the obtained results, it was demonstrated that halophytic ornamental plants like Limonium sp. can adapt to soils with extreme salinity.

Key words: *cultivation substrates, halophytes, salinity, ornamental plant, cut flowers.*

INTRODUCTION

Agriculture plays an important role in the economy of the most countries of the world. Salinity, as an effect of the climate changes, is increasingly affecting agricultural areas by-9% globally and by 11-17% within Europe (Zhang & Cai, 2011), significantly diminishing production (Qados, 2011; Estrada et al., 2021), the income and implicitly the living standard of the population.

The increasing impact both of climate change and unsustainable irrigation practice (Stavridou et al., 2017) imposes challenges for the valorisation of such salt affected areas (Bimal and Harun, 2017).

One solution could be the use of ornamental plant species, halophytes or moderately halophytes, for soil remediation and ecosystem services quality improvement and, also for ornamental horticulture that require a large consumption of water, for flower production (Francisco et al., 2017; García-Caparrós & Lao, 2018).

Apart from the established ornamental plants, new ornamental crops for cut flowers are often introduced. Once they leave the natural environment, they become real trends on the international markets. Their migration from one country to the other begins as early as the 18th century (Darras, 2021).

In the context of CO_2 footprint restrictions and global warming, the cut flowers production may be affected. At the same time, considering public demand for cleaner agricultural products, sustainable cultivation of ornamental plants species (cut flowers) can be an ecological alternative option. Considering these factors, it is useful to know which species are most suitable for cultivation on saline soils.

According to Bellache et al. (2022), *Amaranthus* species proved to be salt tolerant in all developmental stages. This ability being given by the stomatal morphology, respectively: low density, aperture, and basal conductance. In *Amaranthus tricolor* it was observed an increase of carotenoids and antioxidant enzymes, such as superoxide dismutase (SOD). *Limonium*

sinuatum also have been proofed to have salt tolerant ability, in the laboratory resisting up to a sodium chloride (NaCl) concentration of 400 mM (Guo et al., 2022).
In this context, the paper aims to present data related to the behaviour of ornamental plant species in order to state the hypothesis according to which they can growth on sever saline soil.

MATERIALS AND METHODS

The study was carried out in the Research Greenhouse and Research Center of Food Quality and Agricultural Products - USAMV of Bucharest, during the period January-July.

Plants varieties

The varieties and species used in this experiment were: *Celosia plumosa* 'Fresh look mix', *Celosia plumosa* 'Bombay Cherry', *Celosia plumosa* 'Ice Cream (Mix)', *Celosia plumosa* 'Glorious Red', *Limonium sinuatum* 'QIS mix', *Amaranthus* 'Autumn Palette', *Amaranthus caudatus*, and *Gypsophila elegans* 'Crimson'. The seeds were achieved from national market (Anthesis International S.R.L.).

Soil types

With regard to the cultivation substrates, six variants of cultivation were used as follows: S1, S2, S3, S4, S5, S6 having different electrical conductivity (EC) values (Table 2).

Electrical conductivity (EC) assay

The EC was realised using an adapted method (Rayment and Higginson, 1992).

Cultivation substrates

The research activities started with the preparation of the cultivation substrates. Thus, the soil was deposited and exposed in a thin layer in order to dry, because it had a very high humidity and could not be manipulated. After drying at room temperature, the soil was crushed for optimal handling and last but not least, in order to establish conditions favourable to the development of seed germination and root system of the studied plants (Figure 1).

Figure 1. Shredding the soil for sowing

Also, dried roots of other species and stones were removed (Figure 2).

Figure 2. Sifting the soil for sowing

Cultivation substrates for germination test

Experimental variants

To study the behaviour of the seeds in the cultivation substrate, the following cultivation substrates were used (Figure 3).

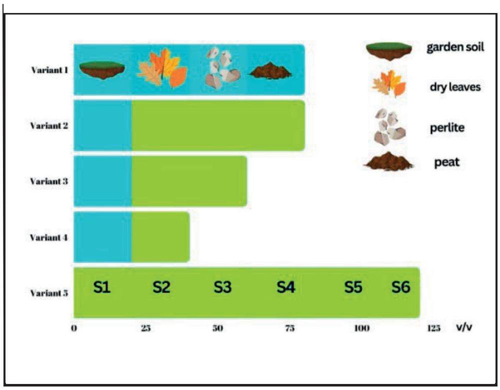

Figure 3. The composition of cultivation substrates (v/v)

Experimental variants for germination test

Experiment 1

To study the behaviour of the seeds in the cultivation substrate, the following cultivation variants were used (Figure 4).

The experiments started by sowing 50 seeds of each *Celosia plumosa* 'Fresh look mix', *Celosia plumosa* 'Bombay cherry', *Celosia plumosa* 'Ice Cream (Mix)', *Celosia plumosa* 'Glorious Red', *Limonium sinuatum* 'QIS mix' in V1 to V5 cultivation substrates.

In the climatic chamber, the trays with seeds were germinated at a temperature of 18-20°C for a period of 8-11 days, photoperiod 10/14 h depending on the indications of the seed manufacturer.

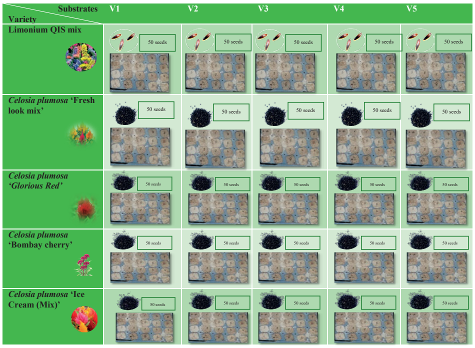

Figure 4. The experimental variants of seeds germination test

Experiment 2

Since the germination percentage of the seeds in the case of experiment I was much lower than that registered by the manufacturer, *Amaranthus* sp. 'Autumn Palette', *Amaranthus caudatus*, and *Gypsophila elegans* were tested within experiment 2.

Therefore, 3-4 seeds were placed in each peat pellet so that a minimum number of 20 plants could be covered per sampling substrate, in order to obtain the seedlings. After 20 days from sowing, having an average size of 2.5 cm, each seedling with a peat pellet was transferred to pots, containing saline soil (S1 to S6).

Thus, a total of 1200 seeds were sown in peat pellets in order to obtain seedlings. After obtaining them, they were transferred to the pots containing the soil of interest S1, S2, S3, S4, S5, S6, in the greenhouse, in an open system, with temperature, photoperiod controlled and irrigated daily. The experimental design is presented in Figure 5.

Figure 5. Experimental design for growth and development test of ornamental species

Statistical analysis

A general linear model, the Duncan test was used for the comparison of the data between groups, using Statistical Package for Social Science (SPSS version 21.0). Statistical processing was performed for a 95% probability. The results are expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Results regarding Experiment 1. As part of the experiment, we wanted to test the seeds germination on the cultivation substrates V1-V4. The following results were obtained, expressed as germination % (Table 1).

Table 1. Germination test on V1-V5 substrates

Plant	V1	V2-V5
Celosia plumosa 'Fresh Look Mix'	84%	*nd*
Celosia plumosa 'Bombay cherry'	22%	*nd*
Celosia plumosa 'Ice Cream (Mix)'	32%	*nd*
Celosia plumosa 'Glorious Red'	42%	*nd*
Limonium sinuatum 'QIS mix'	42%.	*nd*

Although ornamental plants may represent a solution for many fields of interest, the specialized scientific literature is scarce, within the article it was difficult to associate the data obtained with other related studies.

Positive results were obtained only for cultivation V1, the other variants (V2-V5) being negative. Thus, the varieties with the best results were *Celosia plumosa* 'Fresh look mixi' 84%, and *Limonium sinuatum* 'Qis mix' 42% germination percent and were subjected to further studies in the sense of behaviour on the soil with different salinity.

With regard to the germination percentage, there are studies explaining that salinity may influence the process (Yildirim et al., 2002) either by creating an osmotic potential, or the toxic effects of sodium (Na^{+}) and chlorine (Cl^{-}) ions (Khajeh-Hosseini et al., 2003). Salinity can inhibit or delay seed germination (Almansouri et al., 2001) and reduces the growth of seedling depending on cultivar.

So obtained, the seedlings were transferred to 10 cm diameter pots, containing soil with a known salinity. The plants were monitored for a period of 4 months.

The impact of plant cultivation on soil EC value is presented in Table 2. Soil salinity level is site specific and based on electrical conductivity value (Bimal and Harun, 2017). From the results presented in Table 2 it can be seen that for each type of plant, a decrease in the electrical conductivity value.

Table 2. Initial and final electrical conductivity (EC) of the soil

Soil samples	Initial EC/ (mS/cm)	Plant name	Final EC (mS/cm)
S1	4,310	*Amaranthus* sp. 'Autum Palette'	4,080
		Limonium sinuatum 'QIS mix'	2,710
		Amaranthus caudatus	2,560
		Reference (Without plant)	2,540
S2	12,330	*Amaranthus* sp. 'Autum Palette'	13,410
		Limonium sinuatum 'QIS mix'	11,730
		Amaranthus caudatus	11,230
		Reference	15,900
S3	8,050	*Amaranthus* sp. 'Autum Palette'	10,430
		Limonium sinuatum 'QIS mix'	3,270
		Amaranthus caudatus	7,010
		Reference	4,500
S4	5,760	*Amaranthus* sp. 'Autum Palette'	12,240
		Limonium sinuatum 'QIS mix'	12,280
		Amaranthus sp. *caudatus*	10,500
		Reference	7,280
S5	18,630	*Amaranthus* sp. 'Autum Palette'	23,800
		Limonium sinuatum 'QIS mix'	16,900
		Amaranthus caudatus	18,310
		Reference	21,720
S6	24,600	*Amaranthus* sp. 'Autum Palette'	21,190
		Limonium sinuatum 'QIS mix'	19,190
		Amaranthus caudatus	20,490
		Reference	21,810

In the case of S1, the lowest electrical conductivity was recorded after cultivation of *Amaranthus caudatus*, followed by *Limonium sinuatum* 'QIS mix' and *Amaranthus* sp. 'Autum Palette'. In the case of S2, where the salinity was higher than S1, the decrease of electrical conductivity was in the following order: *Amaranthus* sp 'Autum Palette' > *Amaranthus caudatus* > *Limonium sinuatum* 'QIS mix'.

Considering the best-performing species, it can be argued that both *Amaranthus caudatus* and *Limonium sinuatum* 'QIS mix' were positive in terms of diminishing salinity. However, by comparing the species in the same sample (S1 or S2), there are no differences, both of them may be recommended for this purpose.

Also, in the experiment, the influence of plant growth on the change of soil pH value was observed.

Thus, the initial and final (after plant cultivation) pH value for S1-S6 soils were recorded.

The results are shown in Table 3.

Table 3. Soil characterization (pH)

Soil sample	pH(i)	pH(f)	t°C
S1	8.73	8.73	22.3
S2	8.47	8.23	22.2
S3	8.72	8.72	22
S4	8.82	8.66	22.2
S5	8.13	8.07	22.7
S6	8.22	7.98	22.3

According to the results presented above, it can be seen that the pH value was not altered, the soil remaining slightly alkaline.

In order to see if plants can adapt to soil with the salinity shown above, the plants have been monitored, meaning that biometric measurements have been performed. Thus, the height of each plant type was monitored for a period of 4 months.

For each species, 20 pots were monitored. The same varieties and number of plants for S1 to S6 soil types were used.

For S1 variant, the results obtained are presented in Table 4.

Table 4. Plant monitoring for soil type S1

Soil sample	**Plants**	**Plant height (cm) during four months (M)**			
		M1	**M2**	**M3**	**M4**
S1	*Amaranthus sp.* 'Autum Palette'	4.16 ± 0.61^{a}	9.36 ± 1.38^{b}	14.02 ± 1.96^{c}	20.64 ± 2.45^{d}
	Limonium sinuatum 'QIS mix'	3.02 ± 0.68^{a}	6.58 ± 1.55^{b}	11.62 ± 2.21^{c}	11.46 ± 1.79^{c}
	Amaranthus caudatus	1.48 ± 0.31^{a}	3.02 ± 0.55^{b}	7.4 ± 2.18^{c}	14.4 ± 3.7^{d}
	Gypsophila elegans 'Crimson'	1.99 ± 0.39^{a}	3.9 ± 0.82^{b}	6.92 ± 1.28^{c}	10.1 ± 2.07^{d}
	Celosia plumosa 'Glorious red'	0.55 ± 0.13^{a}	1.14 ± 0.13^{a}	1.72 ± 0.64^{b}	3.23 ± 1.12^{c}

*The values are expressed as mean ± standard deviation.

According to the results obtained on the cultivation soil S1, it can be observed that for the species *Amaranthus* sp. 'Autumn Palette' the plant height changed by approximately 5 cm per month, up to a maximum of 20.64 cm.

Also on the same substrate, it can be observed that for the species *Limonium sinuatum* 'QIS mix', the plant height changed on average by 3 cm per month, up to a maximum of 11.62 cm. Regarding this species, similar values (because in month4 some of the plants were affected by salinity) were recorded in months 3 and 4, a sign that the plant has stagnated in development. Regarding the species *Amaranthus caudatus*, the plant height varied throughout the experiment, reaching a maximum value of 14.4 cm. Regarding the species *Gypsophila elegans* 'Crimson', the plant height varied from one month to another, with the plant growing up to 10 cm. Last but not least, *Celosia plumosa* 'Glorious red' recorded changes in height growth, reaching a maximum value of 3.23 cm.

In the experiment carried out on the S1 substrate, the lowest values were recorded in the case of the *Celosia plumosa* 'Glorious red', while the highest plant height values were recorded in the case of the *Amaranthus* sp. 'Autum Palette'.

For S2 variant, the results obtained are presented in Table 5.

Table 5. Plant monitoring for soil type S2

Soil sample	**Plants**	**Plant height (cm) during four months (M)**			
		M1	**M2**	**M3**	**M4**
S2	*Amaranthus* sp. 'Autum Palette'	1.00 ± 0.12^{a}	2.00 ± 0.46^{b}	nd	nd
	Limonium sinuatum 'QIS mix'	1.54 ± 0.36^{a}	3.18 ± 0.62^{b}	3.03 ± 0.55^{b}	nd
	Amaranthus caudatus	0.68 ± 0.22^{a}	1.06 ± 0.5^{a}	4.22 ± 0.76^{b}	4.7 ± 0.58^{b}
	Gypsophila elegans 'Crimson'	0.9 ± 0.26^{a}	1.87 ± 1.00^{b}	2.50 ± 0.0^{c}	nd
	Celosia plumosa 'Glorious red'	0.5 ± 0.0^{a}	1.00 ± 0.0^{a}	2.13 ± 0.0^{b}	nd

*The values are expressed as mean ± standard deviation.

According to the results obtained on the cultivation soil S2 (Table 5), it can be observed that *Amaranthus* sp. 'Autum Palette' species developed in the first two months of cultivation at the pot level. Also, on the same substrate, it can be observed that for *Limonium sinuatum* 'QIS mix' the height of the plant changed on average by 2 cm per month, up to a maximum of 3.18 cm. Regarding this species, similar values were recorded in months 2 and 3 (due to the appearance of some affected plants). The growth and development of the plant took place until the 3rd month. Also in this sense, *Amaranthus caudatus* developed throughout the experiment, reaching a maximum value of 4.7 cm, the values being similar in the last two months. Regarding *Gypsophila elegans* 'Crimson', the plant height varied until the third month, with the plant growing up to 2.5 cm. *Celosia plumosa* 'Glorious red' registered slight changes in height growth, reaching a maximum value of 2.13 cm, similar to those of the *Gypsophila elegans* 'Crimson'. Both *Gypsophila elegans* 'Crimson' and *Celosia plumosa* 'Glorious red' grown until the third month of the experiment.

In the experiment carried out on the S2 substrate, all species recorded lower values than those obtained on S1, *Amaranthus caudatus* being present during the 4 months.

For S3 variant, the results obtained are presented in Table 6.

Table 6. Plant monitoring for soil type S3

Soil sample	Plants	Plant height (cm) during four months (M)			
		M1	M2	M3	M4
S3	*Amaranthus* sp. 'Autum Palette'	3.34 ± 0.44^{a}	7.38 ± 1.15^{b}	10.24 ± 1.15^{c}	13.60 ± 1.13^{d}
	Limonium sinuatum 'QIS mix'	3.94 ± 0.09^{a}	8.72 ± 0.24^{b}	11.34 ± 0.79^{c}	11.06 ± 0.78^{c}
	Amaranthus caudatus	2.64 ± 0.84^{a}	5.78 ± 2.11^{b}	9.18 ± 3.54^{c}	14.10 ± 5.35^{d}
	Gypsophila elegans 'Crimson'	1.70 ± 0.29^{a}	3.84 ± 0.35^{ab}	5.78 ± 0.91^{b}	8.08 ± 1.04^{c}
	Celosia plumosa 'Glorious red'	0.86 ± 0.24^{a}	0.98 ± 0.11^{a}	2.68 ± 0.43^{b}	4.14 ± 0.54^{c}

*The values are expressed as mean ± standard deviation

From the results obtained on the cultivation soil S3, it can be seen that for *Amaranthus* sp. 'Autum Palette' the height of the plant changed by approximately 3 cm per month, up to a maximum of 13.6 cm. Also, on the same substrate, it can be observed that for *Limonium sinuatum* 'QIS mix', the height of the plant changed on average by 4 cm per month, up to a maximum of 11.34 cm. Regarding this species, in months 3 and 4, the height recorded similar values (the small differences come from the plants affected in the last month). In a similar manner to the S1 substrate, the *Amaranthus caudatus* developed progressively throughout the experiment, reaching a maximum value of 14.10 cm. Regarding *Gypsophila elegans* 'Crimson', the plant height varied up to the fourth month, with the plant growing up to 8 cm. *Celosia plumosa* 'Glorious red' registered slight changes in height growth, reaching a maximum value of 4.14 cm in the fourth month of the experiment.

In the experiment carried out on the S3 substrate, all species developed during the four months, *Amaranthus caudatus* standing out with the highest values.

For S4 variant, the results obtained are presented in Table 7.

Table 7. Plant monitoring for soil type S4

Soil sample	Plants	Plant height (cm) during four months (M)			
		M1	M2	M3	M4
S4	*Amaranthus* sp. 'Autum Palette'	2.55 ± 0.71^{a}	5.24 ± 1.55^{b}	8.00 ± 1.47^{c}	11.16 ± 1.51^{d}
	Limonium sinuatum 'QIS mix'	2.52 ± 0.44^{a}	5.16 ± 0.73^{b}	8.24 ± 1.84^{c}	8.78 ± 1.68^{c}
	Amaranthus caudatus	0.57 ± 0.10^{a}	0.98 ± 0.31^{a}	3.94 ± 0.84^{b}	5.43 ± 1.27^{c}
	Gypsophila elegans 'Crimson'	0.52 ± 0.19^{a}	0.24 ± 0.15^{a}	1.68 ± 1.09^{b}	2.88 ± 0.63^{c}
	Celosia plumosa 'Glorious red'	0.85 ± 0.05^{a}	1.58 ± 0.13^{a}	4.27 ± 0.67^{b}	nd

*The values are expressed as mean ± standard deviation.

Regarding the behaviour of the plants on the S4 cultivation soil, it can be observed that for *Amaranthus* sp. 'Autumn Palette' the plant height changed on average by 3 cm per month, up to a maximum of 11.16 cm. Also, on the same substrate, it can be observed that for *Limonium sinuatum* 'QIS mix', the plant height changed on average by 3 cm per month, up to a maximum of 8.78 cm. Regarding this species, in months 3 and 4, the plant height recorded similar values. In a manner similar to S2, *Amaranthus caudatus* developed progressively throughout the experiment, reaching small values, up to 5 cm in height. Regarding *Gypsophila elegans* 'Crimson', the plant height varied up to the fourth month, with values up to 3 cm. *Celosia plumosa* 'Glorious red' recorded slight changes in height growth, reaching a maximum value of 4.14 cm in the third month of the experiment.
In the experiment carried out on the S4 substrate, all species developed during the four months, the *Celosia plumosa* species being an exception.
For S5 variant, the results obtained are presented in Table 8.

Table 8. Plant monitoring for soil type S5

Soil sample	Plants	Plant height (cm) during four months (M)			
		M1	M2	M3	M4
S5	*Amaranthus* sp. 'Autum Palette'	0.70 ± 0.10^{a}	0.57 ± 0.29^{a}	nd	nd
	Limonium sinuatum 'QIS mix'	1.36 ± 0.39^{a}	2.82 ± 0.65^{ab}	4.08 ± 1.19^{c}	4.48 ± 1.42^{c}
	Amaranthus caudatus	0.88 ± 0.13^{a}	1.52 ± 0.16^{b}	2.25 ± 0.78^{c}	nd
	Gypsophila elegans 'Crimson'	0.75 ± 0.35^{a}	1.40 ± 0.57^{a}	nd	nd
	Celosia plumosa 'Glorious red'	0.48 ± 0.04^{a}	0.74 ± 0.39^{a}	nd	nd

*The values are expressed as mean ± standard deviation

In the same way, the results obtained on the cultivation soil S5 (Table 7), can be observed that the species *Amaranthus* sp. 'Autumn Palette' did not develop after the first month of cultivation, the average for the second month is small due to the fact that part of the plants taken in the study stopped growing. Also, it can be observed that for the species *Limonium sinuatum* 'QIS mix' the plant height changed on average by 1.5 cm per month, up to a maximum of 4.48 cm. Regarding this species, in months 3 and 4, the height recorded similar values. In the case of S5, *Amaranthus caudatus* developed reaching small values up to 2 cm in height. In the last month no measurements could be made, due to plant growth stopping.
Regarding *Gypsophila elegans* 'Crimson' and *Celosia plumosa* 'Glorious red', they developed only during the first 2 months of the experiment.
In the experiment carried out on the S5 soil, the only adapted species was *Limonium sinuatum* 'QIS mix'.
For S6 variant, the results obtained are presented in Table 9.

Table 9. Plant monitoring for soil type S6

Soil sample	Plants	Plant height (cm) during four months (M)			
		M1	M2	M3	M4
S6	*Amaranthus* sp. 'Autum Palette'	1.00 ± 0.12^{ab}	2.00 ± 0.46^{b}	3.03 ± 0.55^{b}	nd
	Limonium sinuatum 'QIS mix'	1.54 ± 0.36^{a}	3.18 ± 0.62^{b}	4.22 ± 076^{c}	4.70 ± 0.58^{c}
	Amaranthus caudatus	0.68 ± 0.22^{a}	1.06 ± 0.50^{a}	2.25 ± 0.78^{b}	nd
	Gypsophila elegans 'Crimson'	0.90 ± 0.26^{a}	1.87 ± 1.00^{a}	nd	nd
	Celosia plumosa 'Glorious red'	0.50 ± 0.00^{a}	1.00 ± 0.00^{a}	nd	nd

*The values are expressed as mean ± standard deviation.

Last but not least, according to the results obtained on the cultivation soil S6, it can be observed that for the species *Amaranthus* sp. 'Autum Palette' the plant height changed on average by 1 cm per month, until the third month, reaching a maximum of 3,03 cm. Also, on the same soil, it can be observed that for the species *Limonium sinuatum* 'QIS mix' the results obtained are similar to those of the S5 substrate. In a similar manner to S5, *Amaranthus caudatus* slowly grew, reaching values of up to 2 cm, in the last month the plant did not survive. Regarding *Gypsophila elegans* 'Crimson' and *Celosia plumosa* 'Glorious red', developed only in the first 2 months of the experiment. Similar to the results obtained on the S5 soil, the only adapted was *Limonium sinuatum* 'QIS mix'.

In Figure 6 are presented the results related to the number of leaves according to the species and soil.

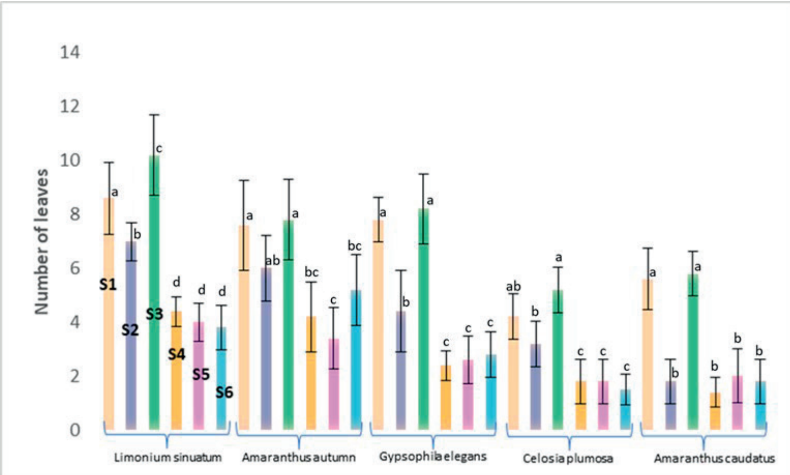

Figure 6. The number of leaves depending on the species and the type of soil
*The values are expressed as mean of five plants ± standard deviation

Regarding *Limonium sinuatum* 'QIS mix', the number of leaves was high in the case of S3, S1 and S2. The other species followed the same pattern, the difference between them being consisted of the smaller number of leaves.

Also, another very important aspect is related to the survival rate of the plants, namely: on the S1 soil, all the plants survived until the fourth month, except the *Amaranthus caudatus* species (month three); on the S2 soil, all the plants survived until month three, with the exception of *Amaranthus caudatus* (month four). On the substrates S3, S4, S5 and S6 both *Amaranthus* sp, and *Limonium sinuatum* 'Qis mix' developed leaves until the fourth month. As for the *Gypsophila elegans* 'Crimson', developed well until the fourth month on S3, S4 and until second month on S5 and S6. *Celosia plumosa* 'Glorious red' developed until month four on S3 and month three on S4 and stopped evolving on S5 and S6.

In the figures below (Figures 7-12), the behaviour of the studied species on each cultivation substrate (S1, S2, S3, S4, S5, S6) is showed.

As can be seen, they are in accordance with the results presented in the tables, being of increased visual impact. Thus, we demonstrated the hypothesis according to which saline substrates can be utilized for cultivation of ornamental plants.

Figure 7. Growth and development of plant species on S1

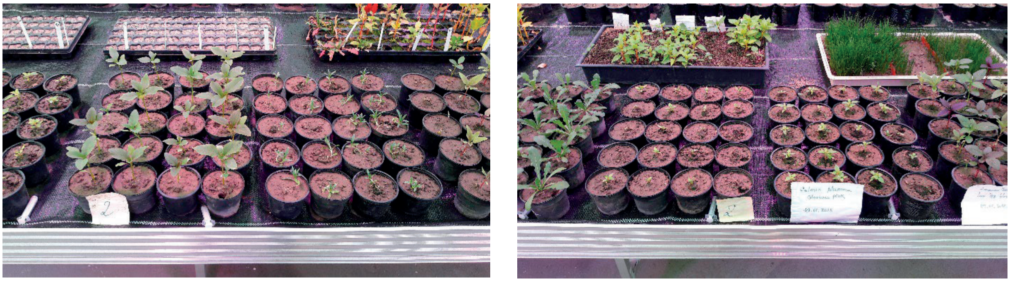

Figure 8. Growth and development of plant species on S2

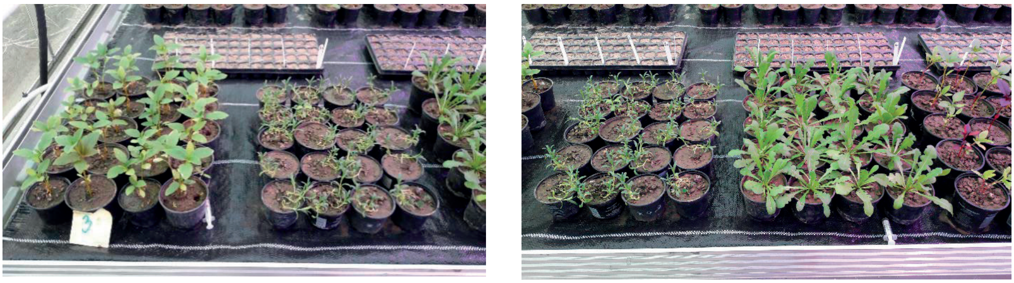

Figure 9. Growth and development of plant species on S3

Figure 10. Growth and development of plant species on S4

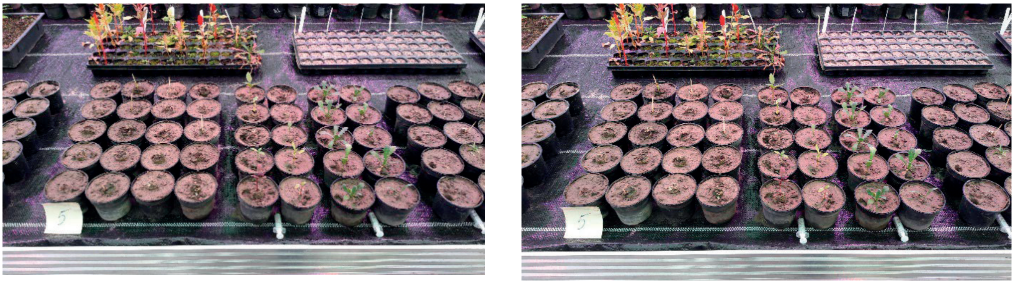

Figure 11. Growth and development of plant species on S5

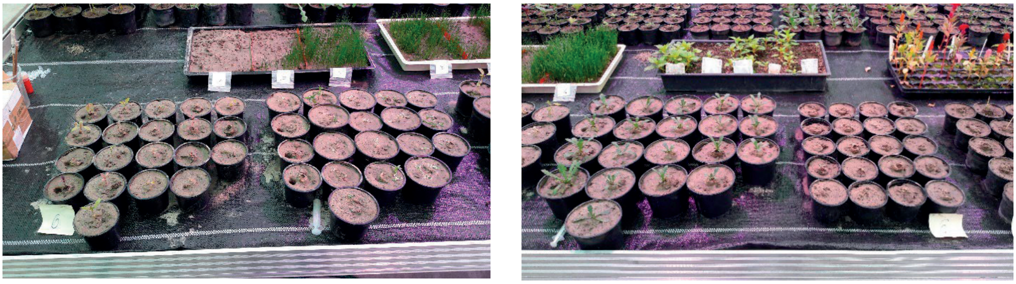

Figure 12. Growth and development of plant species on S6

Although the literature is scarce in this domain, there were several studies referring on the impact of substrate on plant architecture in roses bushes in order to improve the visual impact (Garbez et al., 2018). Other research focuses on the pharmacological part of ornamental plants such as *Celosia cristata* and *Celosia argentea* (Tang et al., 2016).

CONCLUSIONS

Regarding seed germination of studied species, high values were recorded for *Celosia plumosa* and *Limonium sinuatum* 'QIS mix'. With regard to the obtained results, it was demonstrated that halophytic ornamental plants can adapt to soils with extreme salinity, provided that they have been cultivated starting from seedlings. Also, in the same sense, the extraction role of the plants was demonstrated, through the changes made on the electrical conductivity. In terms of electrical conductivity, it has revealed *Amaranthus caudatus* and *Limonium sinuatum* 'QIS mix'. species.
As far as the value of pH is concerned, apparently there may not be a linkage between it and the cultivation of the species used in this experiment.

Through the research carried out, we enhance the studies related to ornamental halophytic species. Also, from the obtained results we may say that ornamental species can be used successfully in the valorisation of non-agricultural soils.

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GAS EXCHANGE AND LEAF CHLOROPHYLL ESTIMATES OF SOME DECIDUOUS TREE SPECIES DURING AUTUMN SENESCENCE

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Abstract

The ontogenetic cycle of the plant involves going through the phases of seeds, seedlings, juvenile, maturity, and senescence. Leaves senescence of woody plants represents a physiological phase of transition from the nutrient's assimilation to their remobilization to different plant' organs, to ensure the survival of the species and its growth in the following year. The purpose of the present study was to quantify the leaves gas exchanges (photosynthesis rate, transpiration rate, stomatal conductance, and intercellular carbon dioxide content) and to estimate their chlorophyll content, during the autumn senescence, in some deciduous tree species (belonging to 16 different families), grown in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The obtained data can be added to those already existing, to better understand the plants behaviour during leaves senescence.

Key words*: botanical garden, physiology, deciduous trees, leaf senescence.*

INTRODUCTION

The ontogenetic cycle of the plant involves going through the phases of seeds, seedlings, juvenile, maturity, and senescence. For individual trees development there are distinguish the following ontogenetic stages: seed, seedling, juvenile, immature (two subgroups), virginal (two sub-groups), young generative, mature generative, old generative and senile (Gatzuk et al., 1980, cited by Evstigneev and Korotkov, 2016).In addition to the ontogenetic cycle of the whole plant, the growth development and trait characteristics of leaves, as the main organs involved in the realization of photosynthesis and respectively the provision of primary products in the ecosystem, directly impact the good functioning of plants within an ecosystem and their behaviour in relation to changes in environmental factors.

If we generally refer to the leaf's senescence, but especially in the case of woody species (ornamental in the urban environment or those in forests), this physiological phase of transition from the assimilation of nutrients to their remobilization (Guo et al., 2021) must be seen mainly as a mean of recycling substances and energy to different plant organs (Li et al., 2020). It is an essential process for plants' fitness (Guo et al., 2021), which ensures the survival of the species and permits new ensuring growths in the following year. Extending the period of maintaining the leaf green colour can be associated with ensuring the continuity of the carbon dioxide assimilation process from the atmosphere and consequently with a translocation of the photo assimilates to the storage organs. At the same time, there is also the possibility of not achieving an efficient resorption of the substances from the leaves before their abscission. Ensuring carbon and nutrient reserves is all the more necessary for species with deciduous leaves, as opposed to those with persistent leaves, because the former must overwinter and start a new growing season without leaves (Piper, 2020).

Considering the transition from the assimilation of nutrients to their remobilization, the recycling of nitrogen compounds resulting especially as a result of the damage of photosynthetic proteins (to amino acids, amides, ammonium) during the disintegration of chloroplasts (organelles containing over 70% of the total protein content of the mesophyll cells) (Fu et al., 2022) provides an important source of nitrogen that plants will assure as a nutrition supplement for growing organs (e.g., new leaves) and seeds (Masclaux-Daubresse et al., 2010). In this sense, the studies of Rolny et al. (2011) suggest that the massive leakage of electrolytes that is registered in the case of senescent leaves can be rather associated with the degradation of proteins and the accumulation of ammonium, than with the damage of the membranes as noted by different authors. On the other hand, Tanabe et al. (2022) determined for molecular nitrogen-fixing plants, that nitrogen resorption from senescent leaves was lower than non-fixing ones due to a higher protein content in the leaves of the former. From an aesthetic point of view, the coloring in different shades of the leaves of ornamental trees (urban environment) or from forests (depending on the species) in autumn, during the senescence period, contributes to spectacular autumn scenery (Lee et al., 2003; Li et al., 2020).

The changes regarding the phenology of the leaves, the dynamics of senescence and their fall are of interest not only from a fundamental, physiological point of view, but also for ecological studies, analysis of the carbon cycle in nature (including those regarding the impact of global climate changes on tree species) and prediction of future carbon uptake (Maschler et al., 2022), or why not, from a tourist point of view. In this context, G´omez et al. (2021) analyzed the dynamics of senescence in Spanish beech forests using time series satellite data between 2001 and 2017, as a cost-effective way of working, which can be used by local and regional authorities.

One of the ways of conserving plants and ensuring genetic resources is ex situ conservation, as is the case with botanical gardens, with the focus of studies on morphological characteristics, reproductive technology and conservation value (Sun et al., 2022). They represent a way of protecting living plants, but at the same time they have important roles in environmental beautification, cultural leisure, and climate regulation. For example, even if the natural habitat of the tulip tree species (*Liriodendron tulipifera* L.) is the mountain forests of North America, its leaves have a special ecological plasticity, it can be successfully cultivated in botanical gardens and parks anywhere in the world, thanks to the mechanisms adaptability of the plant (from the point of view of biochemical, physiological, structural, morphological processes) to fluctuations in environmental conditions (Akinshina et al., 2020).

Therefore, the purpose of the present work was the analysis of gas exchanges and to estimate their chlorophyll content during leaf autumn senescence of different deciduous woody species within the USAMV of Bucharest Botanical Garden, under the conditions of the year 2022. The results obtained can be added to those already existing in the specialized literature (Sun et al., 2022), to better understand the mechanisms of adaptation of plants to the environment in the case of a botanical garden and then to establish artificial management conditions on a scientific basis.

MATERIALS AND METHODS

The experimental site and woody species studied.

The study was carried out in the fall of 2022 on 22 deciduous woody species, belonging to 16 different botanical families (Table 1), grown in the Botanical Garden of the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV of Bucharest), Romania, North latitude of $44^0 24'$ N and $26^0 05'$ East longitude, and an altitude which varies between 60 m and 90 m above sea level, with a temperate-continental climate.

In situ leaf gas exchange

The measurements on the net CO_2 assimilation (Pn) (μmol CO_2 m^{-2} s^{-1}), transpiration rate (Tr) (mmol H_2O $m^{-2} s^{-1}$), stomatal conductance (g_s) (mol H_2O m^{-2} s^{-1}) and intercellular carbon dioxide concentration (Ci) (μmol CO_2 mol^{-1}) were carried out *in situ* (in a sunny day, between 10:00 and 15:00), using 6 cm^2 chamber of the portable photosynthesis system (LCPro-SD - ADC BioScientific Ltd, Hoddesdon, UK) for still green leaves (GL) and yellow senescent leaves (YL)(with green-yellowish or reddish-green shades depending on the species) (at the moment when the leaf detaches from the plant by simple touch) located on the branches at a height of about 2 m from the ground and with southern exposure.

Table 1. Information on the studied species

Plant family names	No.	Scientific name	Common name
Aceraceae (Sapindaceae)	1.	*Acer platanoides* L.	Norway maple
	2.	*Acer pseudoplatanus* L.	sycamore maple
Anacardiaceae	3.	*Cotinus coggygria* Scop.	"smoke tree"
	4.	*Pistacia vera* L.	pistachio
Betulaceae	5.	*Betula pendula* Roth. (*B. verrucosa*)	European white birch
	6.	*Corylus avellana* L.	the common hazel
	7.	*Corylus colurna* L.	the Turkish hazel
	8.	*Carpinus betulus* L.	the common hornbeam
Caesalpiniaceae	9.	*Cercis siliquastrum* L.	Judas-tree
Calycanthaceae	10.	*Calycanthus floridus* L.	Carolina all spice
Cornaceae	11.	*Cornus mas* L.	cornel
	12.	*Cornus sanguinea* L.	red dogwood
Ebenaceae	13.	*Diospyros virginiana* L.	persimmon
Fagaceae	14.	*Quercus rubra* L. (*Q. borealis* Michx.)	red oak or northern red oak
Ginkgoaceae	15.	*Ginkgo biloba* L.	ginkgo or gingko
Lamiaceae	16.	*Vitex agnus-castus* L.	chaste tree
Magnoliaceae	17.	*Liriodendron tulipifera* L.	yellow-poplar, tulip poplar
Moraceae	18.	*Maclura pomifera* (Rafin.) C.K. Schneid.	Osage orange
Oleaceae	19.	*Forsythia x intermedia* Zabel	golden bells
Scrophulariaceae	20.	*Paulownia tomentosa* (Thunb.) Steud.	paulownia
Tiliaceae	21.	*Tilia tomentosa* Moench (*T. argentea* DC.)	silver lime
Ulamceae	22.	*Celtis occidentalis* L.	common hackberry

Leaf chlorophyll content estimation
Relative leaf chlorophyll content (mg dm^{-2}) was estimated using a portable chlorophyll meter (CCM-300, Opti Science, Hudson, USA) that calculates the total chlorophyll content expressed in mg m^{-2}, based on the fluorescence ratio technique.

Statistical analysis
All parameters measured were expressed as means +/- standard errors (SE). Differences between the leaves collected data (Pn, Tr, g_s and Ci between green and yellowing leaves) were supposed to one way analysis of variance (ANOVA), to detect differences of the same species, as well as to do the comparison between the analyzed species. When the data satisfied the requirements of ANOVA, in Excel T-Test has been used to find which samples where significantly different from statistical viewpoint ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

Leaf level gas exchange during autumnal senescence for 22 deciduous woody species is shown in Table 2. As it can be seen, the average value for net photosynthesis (Pn) was around 0.08 µmol CO_2 m^2 s^{-1}, with maximum Pn recorded in paulownia (GL: 8.788 µmol CO_2 m^2 s^{-1} and 5.146 µmol CO_2 m^2 s^{-1}, respectively for YL). At the opposite pole (with the lowest value of Pn) was found *Q. rubra*, GL (-8.76 µmol CO_2 m^2 s^{-1}). For the most studied species, there were no registered significant differences from statistical point of view, between the rate of photosynthesis in GL, compared to yellow ones.
We found a very highly significant differences ($p < 0.001$) in the case of *G. biloba* and *C. coggygria* (higher values for GL) and, respectively, lower values for them in (*Q. rubra* and *C. betulus*) compared to YLs.
Leaf level transpiration rates (Tr) were highly variable ranging between 0.042 mmol H_2O m^{-2} s^{-1} (*C. floridus* - YL) to 5.24 mmol H_2O $m^{-2}s^{-1}$ (*Q. rubra* - GL) and with a general mean value of 0.83 mmol H_2O $m^{-2}s^{-1}$. Comparing done in the case of the species (GL vs YL) emphasized non-significant statistical differences for *L. tulipifera, C. betulus, T. tomentosa* and *P. tomentosa,* respectively *($p \geq 0.05$),* while for the majority of species there have been calculated significantly differences ($p \leq 0.001$) (Table 2).
Stomatal conductance (g_s) mean values were generally very low (even zero mol H_2O m^{-2} s^{-1}) (GL and/or YL). The higher value was only of 0.145 mol H_2O m^{-2} s^{-1} (*Q. rubra* - GL) (Table 2).

Table 2. Gas exchange parameters: (Pn) (net photosynthesis - µmol CO_2 m^{-2} s^{-1}); (Tr) (transpiration rate - mmol H_2O $m^{-2}s^{-1}$; (g_s) (stomatal conductance – mol H_2O m^{-2} s^{-1}) and intercellular carbon dioxide concentration (Ci) (µmol CO_2 mol^{-1}) in leaves of 22 deciduous woody species, in autumn

Species	**Characteristic of the leaf**								**the *p* value and the significance of the differences**			
	Green Leaf (GL)				**Yellowed Leaf (YL)**							
	Pn	**Tr**	**g_s**	**Ci**	**Pn**	**Tr**	**g_s**	**Ci**	**Pn**	**Tr**	**g_s**	**Ci**
A. platanoides	0.04 ± 0.06	0.99 ± 0.07	0.02 ± 0.002	419.4± 9.66	-0.36 ± 0.21	0.35 ± 0.04	0	507± 12.14	0.09 ns	<0.001 xxx	-	0.02 0
A. pseudoplatanus	0.17 ± 0.38	0.83 ± 0.005	0.02 ± 0	419 ± 23.81	-0.39 ± 0.29	0.22 ± 0.007	0	478.2 ± 63.91	0.46 ns	1.74989E-07 xxx	-	0.51 ns
C. coggygria	0.17 ± 0.13	1.75 ± 0.14	0.04 ± 0.003	397.6 ± 4.24	-0.91 ± 0.11	0.61 ± 0.008	0.01 ± 0	504.8 ± 12.35	0.0009 xxx	<0.001 xxx	<0.001 xxx	<0.001 000
P. vera	-0.33 ± 0.10	1.29 ± 0.15	0.026 ± 0.004	441.2 ± 11.85	0.23 ± 0.15	0.84 ± 0.07	0.016 ± 0.002	390.6 ± 9.81	0.01 00	0.0097 xx	0.03 x	0.01 xx
B. pendula	0.89 ± 0.22	2.11 ± 0.10	0.09 ± 0.005	393.2 ± 9.75	0.39 ± 0.05	0.29 ± 0.07	0.01 ± 0	434.6 ± 32.20	0.12 ns	<0.001 xxx	<0.001 xxx	0.27 ns
C. avellana	0.52 ± 0.44	0.22 ± 0.04	0	369.6 ± 14.05	0.30 ± 0.16	0.41 ± 0.02	0	357.8 ± 26.55	0.58 ns	<0.001 000	--	0.66 ns
C. colurna	-0.61 ± 0.07	0.37 ± 0.01	0	530.4 ± 19.19	0.16 ± 0.25	0.26 ± 0.01	0	467.4 ± 15.54	0.02 0	0.0004 xxx	-	0.07 ns
C. betulus	-2.71 ± 0.19	0.79 ± 0.12	0.012 ± 0.0002	654.2 ± 32.32	-0.35 ± 0.06	0.77 ± 0.05	0.01 ± 0	438 ± 5.6	<0.001 000	0.85 ns	0.37 ns	0.003 xx
C. siliquastrum	2.01 ± 1.07	1.36 ± 0.16	0.04 ± 0.004	375.6 ± 29.69	-0.11 ± 0.16	0.33 ± 0.02	0	415.4 ± 28.69	0.17 ns	0.003 xx	-	0.36 ns
C. floridus	2.19 ± 0.82	1.89 ± 0.08	0.04 ± 0.003	357.2 ± 32.73	-0.46 ± 0.55	0.04 ± 0.003	0.01 ± 0.0002	438.8 ± 47.70	0.06 ns	<0.001 xxx	<0.001 xxx	0.69 ns
C. mas	0.29 ± 0.11	0.17 ± 0.02	0	330.6 ± 10.37	0.39 ± 0.13	0.13 ± 0.02	0	299.6 ± 17.69	0.69 ns	<0.001 xxx	-	0.37 ns
C. sanguinea	0.04 ± 0.05	0.13 ± 0.007	0	386.6 ± 24.64	-0.11 ± 0.10	0.65 ± 0.03	0	429.6 ± 12.26	0.32 ns	<0.0001 000	-	0.14 ns
D. virginiana	0.17 ± 0.39	2.95 ± 0.19	0.07 ± 0.005	409.8 ± 9.61	-0.004 ± 0.17	1.34 ± 0.10	0.02 ± 0.002	417.6 ± 11.66	0.73 ns	<0.001 xxx	<0.001 xxx	0.69 ns
Q. rubra	-8.76 ± 0.21	5.24 ± 0.09	0.15 ± 0.004	527.4 ± 2.27	-0.45 ± 0.28	0.84 ± 0.03	0.01 ± 0.002	454.8 ± 9.83	<0.001 000	7.28069E-06 xxx	5.65E-05 xxx	0.004 xx
G. biloba	0.35 ± 0.06	0.23 ± 0.005	0	280 ± 7.86	-0.60 ± 0.11	0.15 ± 0.006	0	575 ± 71.24	0.001 xxx	<0.001 xxx	-	0.02 0
V. agnus-castus	-0.41 ± 0.39	0.91 ± 0.04	0.02 ± 0	445.6 ± 32.80	-0.50 ± 0.12	0.31 ± 0.01	0	510.4 ± 25.77	0.84 ns	9.91203E-05 xxx	-	0.17 ns
L. tulipifera	2.23 ± 0.71	1.74 ± 0.24	0.04 ± 0.008	362.4 ± 19.80	-2.08 ± 0.32	1.86 ± 0.08	0.04 ± 0.003	480.2 ± 11.53	0.002 xx	0.61 ns	>0.05 ns	0.003 00
M. pomifera	0.48 ± 0.18	0.45 ± 0.02	0	317 ± 32.24	-0.36 ± 0.19	0.32 ± 0.02	0	450.4 ± 50.32	0.03 x	<0.001 xxx	-	0.09 ns
F. x intermedia	0.29 ± 0.13	0.29 ± 0.07	0	367 ± 52.12	-0.81 ± 0.37	0.19 ± 0.01	0	587 ± 81.0	0.08 ns	<0.001 xxx	-	0.12 ns
P. tomentosa	8.79 ± 0.63	0.08 ± 0.007	-	-	5.15 ± 0.11	0.07 ± 0.007	-	-	0.004 xx	0.39 ns	-	-
T. tomentosa	-0.62 ± 0.28	0.51 ± 0.04	0.008 ± 0.001	483.8 ± 28.59	-1.07 ± 0.47	0.45 ± 0.008	0.008 ± 0.001	611.6 ± 37.77	0.59 ns	0.24 ns	-	0.14 ns
C. occidentalis	2.01 ± 1.07	1.36 ± 0.16	0.04 ± 0.004	375.6 ± 29.69	-0.11 ± 0.16	0.33 ± 0.02	0	415.4 ± 28.69	0.17 ns	0.003 xx	-	0.36 ns

The data are shown as mean ± SE (n = 5). The comparison was done by the paired T-Test, in Excel, by comparing the values of green and yellow leaves, within the same species. $p \geq 0.05$: There is no statistically significant differences; $p \leq 0.05$: There is statistically significant difference at 95% confidence level; $p \leq 0.01$: There is statistically significant difference at 99% confidence level, $p \leq 0.001$: There is statistically significant difference at 99.99% confidence level.

Intercellular carbon dioxide concentration (Ci) varied between 280 µmol CO_2 mol^{-1} (*G. biloba* - GL) and 654.2 µmol CO_2 mol^{-1} (*C. betulus* - GL). Data presented in Table 2 show that there were not significantly differences between GL and YL ($p \geq 0.001$) with some exceptions, as for example *C. coggygria,* (GL - the mean value of 397.6 µmol CO_2 mol^{-1} as compared with YL -504.8 µmol CO_2 mol^{-1} ($p \leq 0.001$) (Table 2). Total estimated chlorophyll content mean values are shown in Figure 1. As we can see, there are evident significant differences between GL and YL, as well as between the studied species (Figure 1, Table 3). The maximum value has been registered for GL of *A. pseudoplatanus* (7.13 mg dm^{-2}), followed by *T. tomentosa* (7.09 mg dm^{-2}), while the lowest one was noticed for leaves of *G. biloba* (5.75 mg dm^{-2} - GL; 5.46 mg dm^{-2} - YL, respectively).

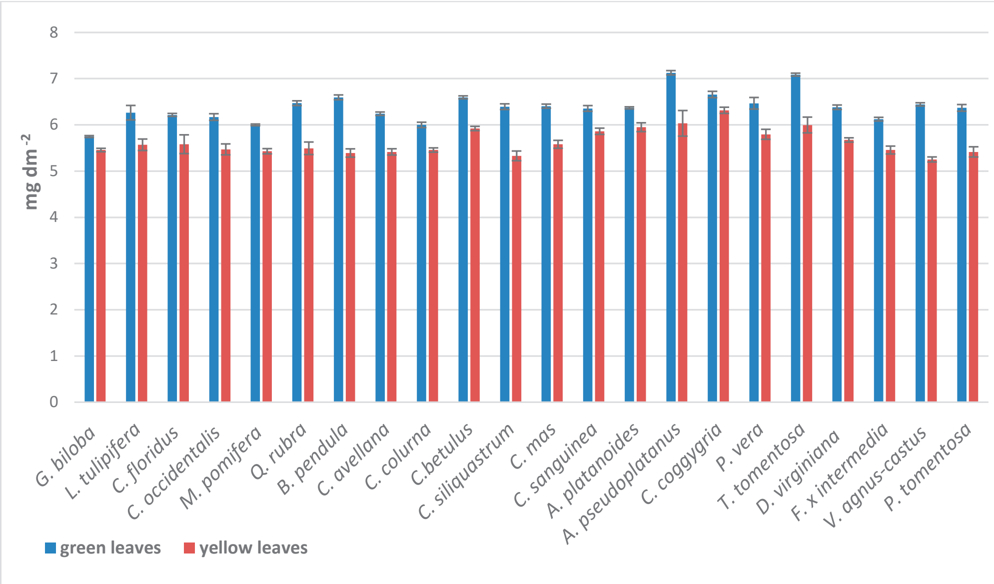

Figure 1. Total chlorophyll content estimated with CCM-300 chlorophyll meter (means ± SE; n=5)

Table 3. The ANOVA: Two-Factor with Replication results showing the effects of species, leaves colour, and their interactions on estimated chlorophyll content

Source of variation	sum of squares (SS)	degrees of freedom (df)	mean square (MS)	F-statistic (F)	*p*-value	critical F-value (F crit)
Sample - Species	14.81397	21	0.705427	16.51289	2.4E-31	1.61629
Columns- Leaf colour	31.44204	1	31.44204	736.0067	8.91E-65	3.894838
Interaction	3.75723	21	0.178916	4.188124	5.41E-08	1.61629
Within	7.51868	176	0.04272			
Total	57.53192	219				

The statistical significance of the differences between total chlorophyll values of the measured tree species was determined by variance analysis (Table 3). Accordingly, between species, as well as between different coloured leaves measurements, there were statistically significant differences between mean values at 99.99 % confidence level (p-value ≤0.001; F-statistic ≥ F critic).

Our study adds to the scientific literature some insights and potential mechanisms in explaining leaf different responses of different species to autumn conditions, in a temperate climate, during plant transition from the active to the dormancy period.

During leaf senescence a nutrient resorption take place with a view to assure a new leaves formation in the next year. Thus, as Yu et al.

(2022) also noticed, duration of the leaf senescence, physiological processes rates in this period and therefore, the efficient mobilization of the nitrogen may affect the growth of leaves in the next season.

Photosynthesis is the most important physiological process specific to autotrophic, green plants. Its intensity depends on the influence of numerous abiotic (e.g., temperature, light, water, carbon dioxide, etc.) and biotic factors. At the same time, the genetic characteristics of the species leave their mark on its dynamics and other associated processes (Zhang et al., 2021). Last, but not least, the photosynthetic capacity is an indicator through which the degree of adaptability of plants to a certain habitat can be characterized.

It is possible that the high rate of the net photosynthesis, even if the stomata are closed, is due to an increased capacity to refix the internal carbon dioxide resulting during respiration (and photorespiration), as well as an increase in the resistance of the mesophyll, which reduces the diffusion process of CO_2 from the cell to the atmosphere. This is how it can be explained that the species that have a higher resistance at the mesophyll level and with thicker cell walls can be more efficient in using the internal carbon dioxide resulting from the respiration process (Eckert et al., 2021).

According to the results obtained by Yu et al. (2022), trees reduce their photosynthesis rate and shorten their growing season to trigger senescence and make the nitrogen remobilization process more efficient. However, if senescence sets in too late, the plants can accumulate a greater amount of photo assimilates, but there is a risk of exposure to damage caused by frost, along with the decrease in the storage of nutrients (e.g., nitrogen loss in leaves) necessary to support the growth of leaves in the following season.

We found very low values of Pn at *Q. rubra* which agree with data obtained by Dhillon (2017) who studied the seasonal dynamics of the process whose rate varied by a maximum of +6,325 μmol CO_2 $m^{-2}s^{-1}$ (in May), at a minimum of -0.618 μmol CO_2 $m^{-2}s^{-1}$ (end of October) during senescence.

For the transpiration rate, the trend was similar, the maximum values being recorded also in May (+1,278 mmol H_2O m^{-2} s^{-1}), and at the end of October a significant decrease of it was reached (+0,191 mmol H_2O m^{-2} s^{-1}).

V. agnus-castus was characterized as the most sensitive and vulnerable in forest-steppe conditions of Ukraine, needs constant replenishment, having high water loss due to transpiration, also the lowest intensity of photosynthesis and dark respiration (Levchyk and Levon, 2018).

In our study, in the case of the *G. biloba* (which is not a native species) a faster and more extensive process of yellowing of the leaves was noted, respectively a faster and massive fall of them, compared to the other studied species, where the fall of the leaves was gradual. In previous studies done in Beijing, China, Zhang et al. (2021) reveals the fact that during senescence (autumn), there is a decrease in the chlorophyll content and an increase in the concentration of carotenoids, as well as changes in the rate of photosynthesis, in close relation with changes in environmental factors, but also depending on the variety and the types of scions. At the same time, the authors recorded in October a decrease in the intercellular CO_2 concentration, in the rate of transpiration, as well as in g_s, compared to July.

The colour changes of the leaves during senescence also reflect their nutritional status and, respectively, the degree of nutrients re-translocation. In the case of red-coloured leaves, a more intense translocation process may occur due to the presence of anthocyanins, more resistant to photoinhibition compared to carotenoids, while in green leaves, during senescence, nutrient resorption it was reduced (Zhang et al., 2021).

In the case of some species studied by us, a reddish colouring of the leaves was observed, which may represent a specific characteristic of the species. This may influence the way the physiological processes unfold during senescence, considering including the positive effects that the accumulation of anthocyanins in the leaves can have for the protection of the photosynthetic apparatus in the case of the excess light energy.

Regarding the senescent autumn leaves of the *Cornus stolonifera* species, Field et al. (2001) highlighted the fact that if they are exposed directly to sunlight, they turn reddish-purple, because of the accumulation of anthocyanins in the vacuoles of the palisade tissue cells and were named “red senescing” On the other hand, the leaves positioned in sub-canopy environments did not contained anthocyanins, were pale yellow green in colour and were named as “yellow senescing”

Lee et al. (2003) highlighted the fact that during leaves senescence, 70% of the woody species contained anthocyanins, synthesized *de novo*, even if they did not appear red, in the situation where the chlorophyll is in an amount less than 20 µg cm^{-2}. Also, the nitrogen content was lower in species with red leaves, which means a better nitrogen resorption in such species.

In addition to the environmental beautification, cultural leisure, and climate regulation, Botanical Gardens are an important sector of the *ex situ* plant conservation (Peschardt and Stigsdotter, 2013, cited by Sun et al., 2022) where very different species can be found, which are grown in an environment completely different from the wild one, and for whose care artificial management is applied.

In similar growing conditions, different plant species expressed in most cases similar values of the eco-physiological characteristics. So, for example, the results of the studies of 47 woody species existing in Botanical Garden in Beijing, highlighted the fact that the apparent quantum efficiency (AQY), light compensation point (LCP), dark respiration (Rd) values were not significantly different between different forms of life, while for the light saturation point (LSP), the net photosynthetic rate at light saturation and specific leaf area (SLA) the differences were significant. Thus, in the artificial environment of the botanical garden acclimated to similar environments, and that the environment had a greater impact on the photosynthetic parameters but had little effect on the SLA and chlorophyll (SPAD values) of plant leaves (Sun et al., 2022).

The increased photosynthetic capacity in different environmental conditions and the rapid growth of *Paulownia* trees was attributed by some authors to their use of C_4 type photosynthesis. But, Young and Lundgren (2022) did a careful analysis of the citations that come to support this idea and the conclusion reached was that there is no scientific data to support this statement. However, the author mentions the fact that numerous investment schemes use information on the physiology of the species (including photosynthesis) to legitimize its use for financial investments and offsetting carbon emissions. The authors confirmed that for *P. tomentosa*, the saturation light level was 1000 µmol m^{-2} s^{-1}, CO_2 compensation point at 63.81 µmol mol^{-1}, water use efficiency 104.54 mmol mol^{-1} and carboxylation efficiency 0.05 mol m^{-2} s^{-1}, respectively, consistent with C_3 photosynthesis type.

The high values of the net photosynthesis rate in the case of the species *Liriodendron tulipifera*, compared to the other species studied, agree with data presented by Akinshina et al. (2020). The species is characterized as being a light-loving mesophilic species, and as having a relatively increased ecological plasticity to light and temperature, which leads to the favouring of the photosynthesis process if the leaves are exposed to increased light intensity and high temperature (as were the conditions in October 2022). To this is added the anatomical changes at the level of the leaves, which provide protection against overheating, and which contribute to the efficient regulation of the plant's temperature, simultaneously with a better supply of water, but also the preservation of water in the plant.

The rate of the photosynthesis process depends on the characteristics of the chloroplasts, organelles exposed to a continuous process of acclimatization to environmental conditions, to which are added the changes associated with the development of the phases of the normal development cycle. During the senescence period, because of their disorganization, they constitute an important source of intermediate metabolites, which will be remobilized to the storage tissues (Domínguez and Cejudo, 2021).

In addition, leaf colour is one of the most important characteristics for plants used for landscape and ornamental purposes (Zhang et al., 2022). Genetic studies by Zhang et al. (2022) highlighted the fact that the silencing of two genes (*ChlH* - magnesium chelatase Subunit H and *POLGAMMA2* – POLYMERASE GAMMA 2) may be related to the phenotype of *Forsythia* with yellow leaves, through the direct or indirect influence of genes involved in chlorophyll biosynthesis and development chloroplast.

Also, the studies carried out on *G. biloba*, by Li et al. (2020) brought clarification regarding the elucidation of the mechanisms of leaf colour change during senescence. There was a significant decrease in the expression of genes for the biosynthesis of chlorophyll *b*, an increase in the expression of genes involved in the biodegradation of chlorophyll, as well as the expression of other genes relative to senescence (such as those related to abscisic acid, jasmonic acid, autophagy, or transcription factors - WRKY, and NAC), whereas cytoskeleton-, photosynthesis-, and antioxidation-related genes decreased from the green leaf stage to the yellow leaf stage.

In the conditions of global climate change, due to the positive effect of higher temperatures, an increase in the duration of the growing season is expected, together with the deposition of nutrients and the assimilation of carbon dioxide, while a limitation of the sink could represent a driver of autumn leaf senescence in deciduous trees (Maschler et al., 2022). Krasnova et al. (2022) studied the effects of the heat wave in the summer of 2018 in Europe on different species (coniferous, deciduous, and conifer-broadleaved) and highlighted the fact that coniferous forests were more resistant to thermal shock, possibly due to the adaptation to a lower water content in the soil. On the other hand, the mixed forest (conifer-broadleaved forest) (pine, spruce, birch, clear-cuts) was more strongly affected compared to the pine forest. From a net CO_2 accumulator in 2017, it became a net CO_2 source in 2018, with a three times higher carbon release, a reduction in gross primary production and a decrease of net ecosystem exchange.

In perspective, implementation of genome editing techniques or control of interest gene expression (e.g., CRISPR/Cas9 system) could also be used to modulate leaf senescence, with the aim of improving agronomic traits, both in terms of crop yields and post-harvest quality (Kim et al., 2018).

CONCLUSIONS

Overall, our results suggest that deciduous species belonging to different botanical families, and with different origins have a specifical physiological behaviour during the autumnal senescence in a botanical garden, in temperate climate conditions. Some native species, as well as those known to have a great adaptation capacity to varied environmental factors seems to be more performed as compared with non-native ones.

The negative photosynthesis rate registered may be due to an intensification of the respiration rate with surpass the former one, and this can signify that the catabolism processes that take place in leaves are those which will also represent the start point for a translocation process before leaves fall, to have a better preparing plant for the winter season, with a view to a successful vegetation start at the next season.

On the other hand, sometimes, a high net photosynthesis values are linked with a higher intercellular carbon dioxide concentration produced by the respiration (adding its reassimilation) and which cannot diffuse into the atmosphere because of the very low values of stomatal conductance or even of their closure, in a drought climate and high temperature conditions such as those specifically under current study area.

Chlorophyll content decreasing is a usual leaf process during senescence. First, it depends on the species characteristic features and chlorophyll breakdown can contribute to the plant redistribution of the nitrogen compounds in perennial plant organs.

Our study riches the knowledge of plants adaptation to the changing environmental conditions and helps to establish the scientific conditions of artificial management.

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THE POLLEN MORPHOLOGY OF DIFFERENT *IRIS* L. SPECIES FROM ROMANIA

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Abstract

In order to have proper identification of Iris species, many scientific works are referring to the morphological features of pollen grains as adequate sources of information about the origin and variability concerning the different populations of Iris.
According to several taxonomic works, in Romania are 17 (18) species of Iris. There is a lack of information about the morphology of pollen in Iris species from our country. SEM analyses of pollen from five populations of Iris species from the Botanical Garden of the University of Agronomic Science and Veterinary Medicine of Bucharest revealed that four of them had reticulated exine, respectively Iris germanica, I. pseudacorus, I. variegate, and I. suaveolens and one had gemmated exine - Iris pumila. The pollen morphological description presented in this study may be of systematic significance to Iris species, enabling species distinction.

Key words*: exine; morphology; pollen; Roumanian Iris species; scanning microscopy.*

INTRODUCTION

The pollen grains, as gametophytes, provide protection for male gametes during dispersal, against mechanical and environmental damage. Their particular shape, surface coating, and sculpturing reflect the adaptations of each species during evolution as vectors of grain transport and adhesion to stigma surfaces (Knox, 1984).
The Iris species have a polymorphism due to their flower structure and reproduction type, which can facilitate the emergence of hybrids (Colasante et al., 2021). The diversity of the characters at every level (morphological, cytological, biomolecular, etc.) made the identification of the Iris species a complex task. These issues, are described also by Dembicz et al., 2018, regarding *I. pumila* and was found a high fragmentation and a high genetic diversity across all investigated *I. pumila* populations in the Kherson region, Ukraine.
Therefore, it is important to find the right methods for assessing the origins of Iris species populations. Pollen grains analysis with scanning electron and optic microscopy are among the methods promoted to evaluate the origins and phylogeny of the species (Colasante et al., 2021). Using light and scanning microscopy methods, Dönmez et Pinar (2001) describe pollen clypeata types' morphological peculiarrities in subgenus *Scorpiris* species from Turkic countries. In order to investigate the pollen morphology of *Iris* species in Croatia and to contribute additional data on species with a wider distribution than the European one, Mitić et al. (2013) analyzed pollen grains from 20 species of Iris employing scanning microscopy. The exine ornamentation of 42 cultivars observed with a scanning microscope allowed, the determination of the systematic, with a focus on cross-breeding, inside *I. barbata* species (Zhang et al., 2021). A study presented in 2022 on pollen micromortphological peculiarities has helped to establish a good delimitation between Korean Iris species (Choi et al., 2022). It is clear from the results of this study that pollen exine ornamentation plays a role in the systematics of *Iris* species.
The most recent work on Romanian vascular plants shows 18 species of the *Iris* genus in the country (Sârbu et al., 2013).
There is not much information about the pollen morphology and micromorphology of the

species from Romania. This study focuses on the morphology of pollen grains from some Iris species cultivated in the botanical garden of our university, conducted using scanning microscopy methods.

MATERIALS AND METHODS

The pollen grains analyzed are from five Iris species cultivated in the botanical garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Four out of these five species are found in different habitats in our country (Sârbu et al., 2013): *Iris suaveolens* - rare in xerophytic grasslands and rocky places in the steppe and wooded steppe; *I. pumila* - often, in grasslands, rocky coasts, or grassy rocky ground, from steppe to beech floor; *I. variegata* - common in grasslands, forest fringes, or on sandy soils from steppe to beech floor; *I. pseudacorus* - common in marshes, meadows, or reedy swamps from steppe to beech and spruce floor. *I. germanica* is a cultivated species occasionally found in the wild.
The fresh pollen grains were collected from anthers and dried at room temperature at the beginning of the anthesis. Observations were made on dried grains in the Research Center for Studies of Food Quality and Agricultural Products. The pollen grains were powdered on SEM stubs and observed directly under the scanning electron microscope (SEM) FEI Inspect S 50 using low vacuum mode. All the measurements were made in the dehydrated stage.
The detailed description of the pollen grains follows Halbritter et al., 2018, pollen monography.
The polar axis and equatorial diameter ratio allowed for the classification of pollen into a shape class, according to Rahmawati et al., 2019.
For each parameter, mean values, standard deviation, coefficient of variation, and Pearson correlation at $p \leq 0.05$, were calculated using Microsoft Excel 2019.

RESULTS AND DISCUSSIONS

Iris species disperse their pollen as isolated grains (monads). The pollen grains are monoaperturate and sulcate, with an elongated aperture located distally (Figure 1).

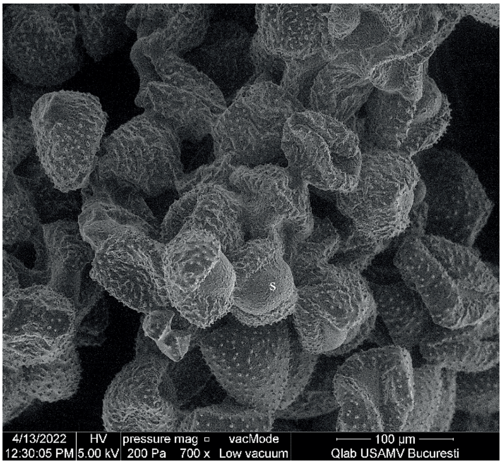

Figure 1. *Iris pumila* - isolate grains, with sulcus (s)

Iris germanica L.
Species of Mediterranean origin with a blooming period of May and June.
In dry conditions, grains are wedge-shaped with a sunken aperture (Figure 2). The ornamentation of exine is of a heterobrochate type, with a reticulated pattern and lumina of different sizes. In each lumina are free-standing columellae.

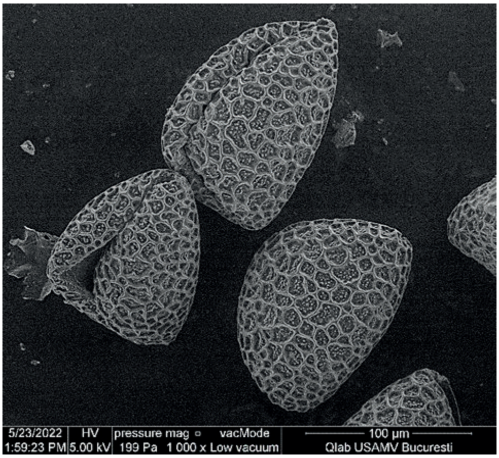

Figure 2. *Iris germanica* - dry pollen grains; free-standing columella in the lumina of the network

Iris pumila L.
Originated in the Ponto-Pannonian-Balkan regions. The blooming period is April to May.
Dry pollen grains are wedge-shaped with a sunken aperture (Figure 1). Exine ornamenttation is a clavate-gemmate type (Figure 3).

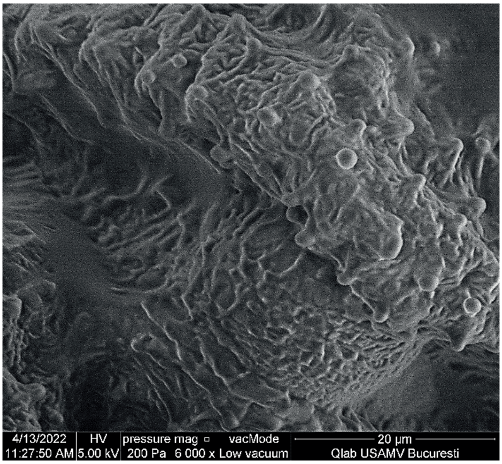

Figure 3. *Iris pumila* - exine ornamentation

Iris pseudacorus L.
Is a European species with a blooming period from May to July.
Pollen grains that are dry are typically boat-shaped with reticulated ornamentation of the exine. Network laminae decrease towards the aperture area (heterobrochate type). The aperture is sunken with infolded edges (Figure 4).

Figure 4. *Iris pseudacorus* - dry pollen grains boat-shaped

Iris suaveolens Boiss. et Reut.
Est Balkan-Anatolian element. Blooming periods last from March to April.
Dry pollen grains are wedge-shaped with heterobrochate exine. There are few free-standing columellae visible (Figure 5).

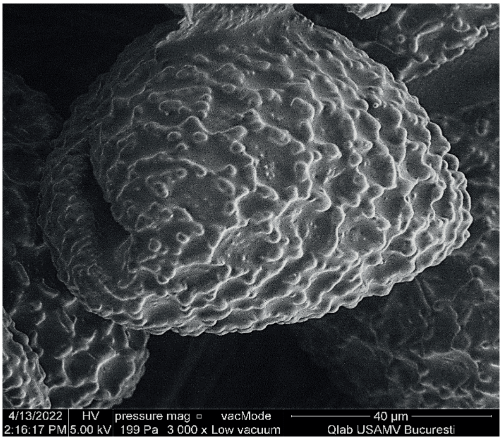

Figure 5. *Iris suaveolens* - heterobrochate exine

Iris variegata L.
Ponto-Central European-Balkan element with the blooming period in May and June.
Dry pollen grains are wedge-shaped. The ornamentation of exine is of a heterobrochate type with free-standing columellae. The aperture is sunken (Figure 6).

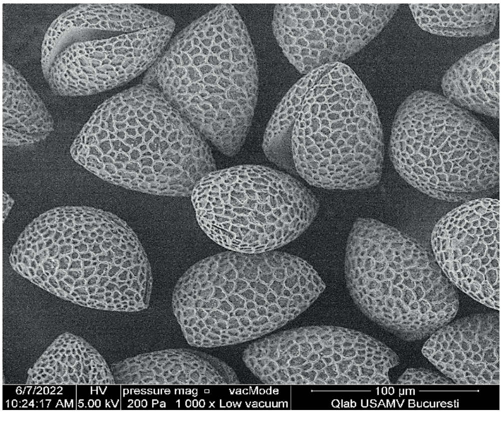

Figure 6. *Iris variegata* - Heterobrochate dry pollen grains

A reticulate pattern of exine was also found in Iris subgenera by Choi et al., 2022, and Dönmez, & IşIK , 2008.
Analyzing the pollen size, a higher polar axis length variability was found in *I. germanica*, followed by *I. pumila*. The lowest variability was found in *I. pseudacorus* species. *I. germanica* presented the highest values from all species, being 41.344 µm higher than *I. pumila*. The dry pollen size varied between 63.860 µm for *I. pumila* to 137.900 µm for

I germanica. For equatorial axis length, higher variability was found in *I. pseudacorus* species, followed by *I. pumila,* and the lowest was found in *I. germanica*. *I. germanica* having the highest equatorial axis length with 35.731 µm more than *I. pseudacorus*. Equatorial axis length varied between 35.77 µm for *I. pseudacorus* and 86.42 for *I. germanica.*

For P/E, also *I. pseudacorus* variety showed the highest variability, followed by *I. pumila*, like in equatorial axis lengths, but the lowest ratio was found in *I. suaveolens*. *I. pseudacorus* presented the highest values, with 1.032 µm then *I. suaveolens*. P/E varied between 0.999 µm for *I. suaveolens* and 3.008 µm for *I. pseudacorus* (Table 1).

Table 1. Pollen morphology of Iris species

Iris varieties	Polar axis length ± SD (µm)	CV%	Equatorial axis length ± SD (µm)	CV%	P/E ± SD (µm)	CV%
Iris germanica	120.797±16.01	13.254	81.933±4.86	5.926	1.478±0.20	13.753
Iris pseudacorus	102.547± 4.90	4.781	46.202±6.78	14.677	2.271±0.40	18.014
Iris variegata	85.003± 7.26	8.547	56.631±4.45	7.861	1.514±0.22	14.367
Iris suaveolens	84.989±6.04	7.110	68.981±6.44	9.340	1.239±0.11	9.151
Iris pumila	79.453±8.04	10.118	60.085±8.45	14.064	1.341±0.18	13.549

SD - standard deviation, CV - coefficient of variation, P/E - ratio of polar axis length to equatorial axis length

I. pseudacorus presented the highest values, with 1.032 µm then *I. suaveolens*. (Table 1).

Our results showed that the Iris species studied have a high pollen shape, equatorial axis, and polar axis length variability.

Analyzing the Pearson correlation, for polar axis length, was found a moderate negative relationship between *I. germanica*, *I. pseudacorus,* and *I. suaveolens*

Table 2. Correlation among *Iris* species and pollen polar axis length

Polar axis length (µm)	*I. germanica*	*I. pseudacorus*	*I. variegata*	*I. suaveolens*	*I. pumila*
I. germanica	1				
I. pseudacorus	0.355	1			
I. variegata	0.369	-0.215	1		
I. suaveolens	-0.600	-0.633	0.290	1	
I. pumila	0.414	-0.151	-0.106	0.080	1

r < 0.05

A very weak positive correlation was found between *I. suaveolens* and *I. pumila* (Table 2).

Regarding equatorial axis length, a strong relationship was found between *I. germanica* variety, *I. variegata,* and *I. pumila*. A strong relationship was found also between *I. germanica* and *I. variegata* (Table 3).

Dönmez & IşiK in 2008, in their study, found that Iris pollen grains were medium to large in size, the measurements varied between 45-163 µm for polar axis length and 33-163 µm for equatorial axis length, our findings are in accordance with their study.

A moderate positive relationship was found between I. *suaveolens* and *I. pumila* related to the ratio between polar axis length and equatorial axis length (Table 4). P/E is an important parameter that could vary depending on the environmental conditions (Güvenet al., 2014).

Table 3. Correlation among Iris species and pollen equatorial axis length

Equatorial axis length (µm)	*I. germanica*	*I. pseudacorus*	*I. variegata*	*I. suaveolens*	*I. pumila*
I. germanica	1				
I. pseudacorus	-0.186	1			
I. variegata	0.787	-0.144	1		
I. suaveolens	-0.441	-0.095	-0.247	1	
I. pumila	0.831	-0.552	0.719	0.162	1

r < 0.05

Table 4. Correlation among *Iris* species and the ratio between polar axis length and equatorial axis length of pollen

P/E (µm)	*I. germanica*	*I. pseudacorus*	*I. variegata*	*I. suaveolens*	*I. pumila*
I. germanica	1				
I. pseudacorus	0.072	1			
I. variegata	0.563	-0.079	1		
I. suaveolens	0.505	-0.612	0.292	1	
I. pumila	-0.002	-0.730	0.497	0.666	1

Wang & Dobritsa, 2018 in their study specified that there is a correlation between pollen surface morphology and plant pollination. It was observed that pollinators can influence the patterns and decorations from pollen surfaces, whereas plants pollinated with the help of wind or water have a smoother pollen surface, making the pollen more aerodynamic. These differences in patterns might influence pollen hydrodynamic and pollen-stigma interaction. Lumina and muri from pollen exine, also have a very important role in differentiation between species.

The measurements on lumina (Table 5) width, showed that *I. suaveolens* have the largest width of 8.406 µm, the largest being of 10.610 µm, and the smallest of 5.690 µm due to heterobrochate exine. Between *I. suaveolens* and *I. germanica*, the values for lumina width, are close, the difference between them being 0.042 µm. *I. pseudacorus* had the smallest lumina of 0.883 µm, the largest being 1.416 µm and the smallest of 0.117 µm, with network laminae decreasing towards the aperture.

I. germanica was found to have the highest length of lumina (11.246 µm), having a maximum length of 14.940 µm and a minimum of 8.378 µm. *I. suaveolens* with 13.790 µm maximum length and with a minimum of 8.014 µm. The smallest lumina was found in *I. pseudacorus* with 2.711 µm the maximum length and 0.989 µm minimum length.

Table 5. Lumina characteristics of Iris species

Iris varieties	Width ± SD (µm)	CV%	Length ± SD (µm)	CV%
I. germanica	8.364±1.494	17.866	11.246±1.999	17.773
I. pseudacorus	0.883±0.339	38.448	1.574±0.496	31.496
I. variegata	4.180±1.081	25.852	7.756±1.674	21.582
I. suaveolens	8.406±1.359	16.169	10.384±1.923	18.514

SD - standard deviation, CV - coefficient of variation

The results, related to lumina measurements, showed that Iris species have irregular-shaped lumina, and we found variability between the studied Iris species.

Our results are in concordance with those of Mitić et al. 2013, that found that on the basis of karyological results, *I. germanica* pollen type derived from the *I. pumila* pollen type, and also *I. sibirica* from Bjelolasica Mountain, have possible parents from the primitive subgenus *Limniris* and the other possible parent is a taxon from the series *Pumilae*, so *Pumilae* can be more primitive.

CONCLUSIONS

Among the studied species we found variations in pollen morphology related to the shape, size, and ornamentation of the exine.

Iris germanica, *I. variegata,* and *I. suaveolens* have the exine heterobrochate with lumina with free-standing columellae, *I. pseudacorus* has the exine heterobrochate, but without free-standing columellae.

I. pumila has the exine ornamentation clavate-gemmate type.

I. germanica was found to have the highest length of lumina, and the smallest lumina length was found in *I. pseudacorus*.

I. germanica presented the highest values for polar axis length and equatorial axis length from all studied species.

I. pumila presented the lowest polar axis length and also the ratio between the polar axis length and equatorial axis length of pollen.

A strong relationship between *I. germanica* variety, *I. variegata,* and *I. pumila*, was found, and also between *I. germanica* and *I. variegata*.

Our study of the morphological aspects of the Iris species pollen, offers an important characterization of the species from our country, helping in their differentiation.

The results of this study bring new information on the characterization of pollen, contributing to conservation and genetic improvement.

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INVASIVE ALIEN PLANTS IN GRASSLANDS FROM THE LAND OF FĂGĂRAȘ

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Abstract

Using flowing water as a transportation medium for seeds and fruit, invasive plant species can spread rapidly, making river banks an environment where they can grow prosperous populations. In the plain of the Land of Fagaras, on the fringe of water courses, are many meadows with rich plant diversity. During field research conducted in 2021 and 2022, data from grasslands near twelve towns were collected to observe the presence of invasive plant species in this area. Sixteen species from the List of invasive and potentially invasive alien plants in Romania were identified in or close to analyzed grasslands. One of them, Impatiens glandulifera Royle, is on the List of invasive alien plants of concern for the EU. In the river banks, small or extended populations are formed, and only a few of these invasive species are installed in the grassland, except for Erigeron annuus.

Key words*: grassland; invasive alien plant species; the Land of Fagaras.*

INTRODUCTION

Invasive alien species are described as non-native species that once introduced outside their natural range have the ability to establish themselves in the recent area, produce offspring, and spread exponentially, extending their range (Pyšek et al., 2004).

The effects of those species on the environment were studied, and they ranged from changes in the richness and abundance of native species and the increased risk of native species extinction to changes in the ecosystems' functioning and services by changing nutrients and contaminating cycling, hydrology, and habitat structure (Pyšek et al., 2020).

The impact of invasive alien species on biodiversity is expected to grow in the future as a result of synergies with other global changes such as climate change, habitat loss, or human pressure (Genoves & Monaco, 2013; Pyšek et al., 2020).

Appropriate management solutions can prevent or attenuate the consequences of biological invasion. Therefore, complete data on the biological features of invasive species and their dispersion in specific habitats are necessary (Anačkov et al., 2013).

The information needed to prevent, control, or limit the spread of invasive species can be provided by compiling accurate lists on local levels (McGeoch et al., 2012).

Sârbu et al. (2022) published a review of the scientific literature on adventive plants, which emphasizes a national pattern regarding plant species invasions, geographical origins, and pathways of introductions. This is an important starting point for the management and action plans for invasive alien plant species in Romania. As this paper concludes that data collection in our country was conducted opportunistically rather than systematically, it is important to sample more intensive areas away from major academic and research facilities, and outside of popular protected areas.

Recently, several scientific papers or theses have addressed the subject of invasive plants in different regions of our country: Skolka & Preda (2011) for the Black Sea coast, Szatmari (2012) for the Carei Plain, Raduțoiu & Băloniu (2021) for the Oltenia region, Gradinaru (2021) for the Agighiol Hills from Dobrogea region or Susanu (2022) for the lower course of the Siret River.

Zimmermann et al. (2015) approach the subject of invasive plant species in the central part of Romania, in the South of Transylvania, around Sighișoara town, sampling a large variety of landscape elements, from the border of the

roads to farming landscapes and abandoned lands. According to them, there are eight species of plant invaders that have the potential to expand their distribution, in connection with landscape heterogeneity, roads, agricultural areas being at the top of colonized habitats.
The Land of Fagăraş, also known as the Land of Olt, is an erosion depression of 1000 km^2 situated in the South of the Transylvania Plateau. It is traversed from east to west by the Olt River. There are extensive terraces and meadows along the Olt and its tributaries, which are occupied by secondary grasslands (Ghinea, 1996; Grecu et al., 2008; Cimpoieş, 2013) alongside oak and beech forests. Only a few scientific papers on flora and vegetation are reported in this region. In 1969, Ularu wrote about the vegetation in hay meadows in the Persani Mountains. Concerning the vegetation of Sinca Noua, there are two works, the first in 1958 (Pop & Terentiu) and the second in 2008 (Danciu et al.). A list of species and habitats was included in the last one. Two invasive alien plants are found between species. In this paper, we describe alien invasive species found in grasslands near twelve towns from the Land of Făgăras, their taxonomy, ways of introduction, and biological characteristics in order to analyze their invasivity and the potential impact on plant diversity.

MATERIALS AND METHODS

The observation was carried out in grasslands near 12 towns in the Land of Fagaras (Figure 1).

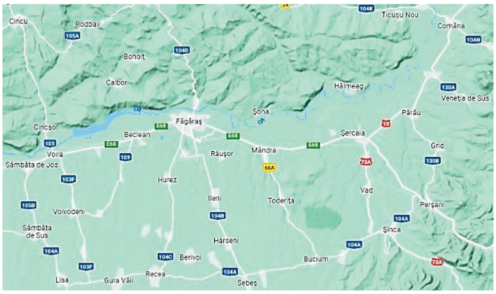

Figure 1. The area of investigation (https://www.google.com/maps/)

A layered alluvial-proluvial plain, formed by the terraces and meadows extended along the Olt valley and its tributaries, represents the relief of this territory (Ghinea, 1989).

The grasslands of the Land of Făgăras searched by us, are near the fringe of water courses, and they are classified in the association of *Molinietalia caerulaea* order such as *Agrostideto stoloniferae-Festucetum pratensis* Soo 1949, *Festuco rubrae-Agrostetum capillaris* Horvat 1951 (Pop, 2008), and *Anthoxantho-Agrostetum capillaris* Sillinger 1933 (Chifu et al., 2014).
Based on the list of alien invasive or potentially invasive plant species in Romania (Anastasiu et al., 2019), the list of plants was made through field observations, in 2021 and 2022. The information about the taxonomy and biological features of species was taken from books of Flora of Romania (Ciocârlan, 2009; Sârbu et al., 2013).

RESULTS AND DISCUSSIONS

The grasslands of the Land of Făgăras, which are abundant in mesophyllous species, are economically, biologically, and historically significant. They are used as hay meadows or as pastures. Several rare species, such as orchids, can be found, some of which are noted in the Red List of Plants of Romania (Danciu et al., 2008). Land stewardship of the local inhabitants and knowledge of the uses of plants in folk medicine, dyeing the wool, or alimentation sustain the historical dimensions of these grasslands (Drăgulescu, 1995). Anthropogenic pressure can affect the floristic composition and increase the risk of invasive species appearing.

Taxonomy, origin, and mod of introduction
We identified 16 species from the List of alien invasive or potentially invasive plant species in Romania (Anastasiu et al., 2019), which are located near or into grasslands. The majority are originally from North America, and only three of them are from Asia (*Impatiens glandulifera*) and South or Central America (*Galinsoga parviflora, Xanthium strumarium*) (Table 1).
Regarding pathways of introduction, some were introduced as ornamental plants and escaped from gardens where they were cultivated (*Oenothera biennis, Impatiens glandulifera, Echinocystis lobata, Solidago canadensis, Solidago gigantea*). Species like *Solidago canadensis* and *Impatiens glandulifera* are still found in gardens (Drăgulescu, 1995).

Acer negundo was also introduced as an ornamental plant and appears in a list of plants in The Bishop's Palace gardens at Fulham in 1692 (Brown, 2004). It was planted in many places in Romania as forestry species.
Species such as *Amaranthus powelli, Erigeron annuus, Galinsoga parviflora*, or *Matricaria discoidea* were introduced into botanical gardens as curiosities from which they emerged and spread in Europe and Asia (Mabey, 1996; Pârvu, 1997).
It is possible that *Ambrosia artemisiifolia* has been introduced accidentally with some cereals (Anastasiu & Negrean, 2007).

Table 1. Taxonomy, origin, and pathways of introduction of alien invasive species in the Land of Fagaras grasslands

Species	Family	Origin	Pathways of introduction
Amaranthus powelii	Amaranthaceae	NAm	Escaped from garden
Amorpha fruticosa	Fabaceae	NAm	Planted ornamental,
Robinia pseudoacacia	Fabaceae	NAm	Planted forestry, sand stabilization
Oenothera biennis	Onagraceae	NAm	Escaped from garden
Acer negundo	Aceraceae	NAm	Planted forestry, ornamental
Impatiens glandulifera	Balsaminaceae	As	Escaped from gardens
Echinocystis lobata	Cucurbitaceae	NAm	Escaped from gardens
Ambrosia artemisiifolia	Asteraceae	NAm	With some cereals
Erigeron annuus/ annuus	Asteraceae	NAm	Escaped from gardens
Erigeron canadensis	Asteraceae	NAm	Unknown
Galinsoga parviflora	Asteraceae	SAm	Escaped from gardens
Matricaria discoidea	Asteraceae	NAm	Escaped from gardens
Rudbeckia laciniata	Asteraceae	NAm	Escaped from gardens
Solidago canadensis	Asteraceae	NAm	Escaped from gardens
Solidago gigantea	Asteraceae	NAm	Escaped from gardens
Xanthium strumarium	Asteraceae	C, SAm	Accidentally

Legend: NAm - North America; C, SAm - Central, South America; As - Asia

After being introduced to Europe, *Robinia pseudoacacia* and *Amorpha fruticosa* were planted in Romania to stabilize sandy soils or along roads (Anastasiu & Negrean, 2007).
Xanthium strumarium, which is known in Europe since the XVI century (Săvulescu et al., 1964), was probably introduced accidentally as a contaminant.
Erigeron canadensis is known to have been introduced into Europe from North America in 1655, but there are no data on its pathways of introduction. Romania's data on the species originates from around 1814 (Săvulescu et al., 1964). Form of life and reproduction mode, pollination, and seeds dispersal type

The success of alien plants in new habitats depends on their flexibility in basic biological processes

Ten of the species are therophyte or hemitherophyte, which reproduce by seeds (Table 2). Some species include *Erigeron canadensis, Erigeron annuus, Oenothera biennis, Impatiens glanduligera, Galinsoga parviflora*, or *Matricaria discoidea* show a long period of flowering, from May to September. Other species - *Echynocystis lobata, Xanthium strumarium, Ambrosia artemisiifolia*, or *Amaranthus powelii*, have a flowering period during the second part of the summer (Sârbu et al., 2013).
The remaining species, which are six in number, are perennial. Three species - *Robinia pseudoacacia, Amorpha fruticosa,* and *Acer negundo* - are phanerophytes, and they reproduce vegetatively through root sprouts in addition to seeds.
The other species are herbaceous with rhizomes. For *Solidago* species, vegetative reproduction is more important than seed production.
Most of the species exhibit an entomophily type of pollination, without specialization on an insect or a group of insects in particular (Table 2). Furthermore, they can perform pollination with two agents, one principal and one

secondary (like wind). It's also possible the self-pollination (Wang et al., 2021).

Seed dispersal allows species to expand their range. Anemochory and zoochory are the preferred methods of dispersing the seeds (Table 2). Autochory is combined with other methods of dispersal, for example to *Galinsoga parviflora* or *Impatiens glanduligera*.

Regardless of the case, antropochory remains a major factor in the spread of invasive species, whether accidentally or intentionally (Warwick & Sweet, 1983).

Table 2. Form of life, reproduction, pollination, and seed dispersal mode of alien invasive species in the Land of Fagaras grasslands

Species	Form of life/ Reproduction mode	Pollination type	Seeds dispersal
Amaranthus powelii	T; sexual (seeds)	Anph/E	An/Aut/Z
Amorpha fruticosa	Ph; sexual (seeds) and vegetative (root sprouts)	E	Aut/ Z
Robinia pseudoacacia	Ph; sexual (seeds) and vegetative (root sprouts)	E	Aut/Z
Oenothera biennis	Th; sexual (seeds)	E	An/H
Acer negundo	Ph; sexual (seeds) and vegetative (root sprouts)	Anph	An/others
Impatiens glandulifera	T; sexual (seeds)	E	Aut/ others
Echinocystis lobata	T; sexual (seeds)	E	Aut
Ambrosia artemisiifolia	T; sexual (seeds)	Anph	Z
Erigeron annuus/ annuus	T; sexual (seeds)	E	An
Erigeron canadensis	T; sexual (seeds)	SP/E	An/ H
Galinsoga parviflora	T; sexual (seeds)	E	An/ Antrop/ Z
Matricaria discoidea	T; sexual (seeds)	E	An
Rudbeckia laciniata	H; sexual (seeds) vegetative (rhizome)	E	An
Solidago canadensis	H; vegetative (rhizome, stolon) sexual (seeds)	E	An
Solidago gigantea	H; vegetative (rhizome, stolon) sexual (seeds)	E	An
Xanthium strumarium	T; sexual (seeds)	Anph	Z

Legend: T - therophyte; Ph - phanerophyte; Th - hemitherophyte; H - hemicryptophyte; Anph - anemophily; E - entomophily; SP - self-pollinated; An - anemochory; Aut - autochory; Z - zoochory; H - hydrochory; Antrop - antropochory.

The frequency, density, and spatial distribution of populations of invasive alien species in the Land of Făgăraș grasslands

The analysis of the populations' frequency in the twelve localities reveals that there are differences between species: only *Erigeron annuus* populations are present in all the observed locations (Table 3). The next species with an obvious presence are *Impatiens glandulifera* and *Oenothera biennis*, whose populations were found in four locations. The remaining species are present in two (*Amorpha fruticosa, Robinia pseudoacacia, Erigeron canadensis, Matricaria discoidea, Xanthium strumarium*), or only one location (*Amaranthus powelii, Acer negundo, Rudbeckia laciniata, Galinsoga parviflora, Solidago canadensis, S. gigantea*).

The population sizes of the *Erigeron annuus* in different locations may comprise between 50-100 individuals, in some places even can be found populations with more than 100 individuals (in a wet grassland near Holbav we found populations made of 100-500 individuals) (Figure 2).

Figure 2. View of *Erigeron annuus* in Valea Lupului meadow

Impatiens glandulifera is the second-largest population, with 51-100 plants found in grasslands near Șinca Nouă (Figure 3).

In certain locations, large populations of *Echinocystis lobata* were found, such as at the edge of grasslands in relation to fringe water courses, like in Comăna de Jos or Grid.

Populations of over 50 plants were found for *Solidago* species, but they are in relation to farming lands that are near the grasslands, in Lisa or Comăna de Jos.

The rest of the inventoried species have small populations. They can range in size from one to 10 plants or up to 50 individuals (Figure 4).

We can talk about three situations regarding the spatial distribution of species: species that are part of grassland associations, like *Erigeron annuus* and *Impatiens glandulifera*; species that are found in disturbed places in grasslands or disarranged meadows, like *Oenothera biennis, Rudbeckia laciniata, Xanthium strumarium, Matricaria discoidea* or *Erigeron canadensis*; plants found in the vicinity of grasslands, towards roads, water courses, or crops around meadows, such as *Galinsoga parviflora, Amaranthus powelii, Ambrosia artemisiifolia, Robinia pseudoacacia, Solidago canadensis, Amorpha fruticosa, Echinocystis lobata,* or *Acer negundo*.

Figure 3. View of Impatiens glandulifera

Figure 4. View of *Oenothera biennis* in Copăcel meadow

Table 3. The frequency of populations of invasive alien species in the Land of Fagăraș grasslands

Species/Town	Șinca Nouă	Hălmeag	Vad	Toderița	Copăcel	Grid	Sebeș	Holbav	Lisa	Perșani	Șercaia	Comăna de jos
Amaranthus powelii	-	-	-	-	-	-	-	-	-	-	+	-
Amorpha fruticosa	-	-	-	-	-	+	-	-	-	-	+	-
Robinia pseudoacacia	-	-	-	-	-	+	-	-	+	-	-	-
Oenothera biennis	+	-	-	-	+	-	+	-	+	-	-	-
Acer negundo	-	+	-	-	-	-	-	-	-	-	-	-
Impatiens glandulifera	+	+	-	-	+	-	-	-	+	-	-	-
Echinocystis lobata	-	+	-	-	-	+	-	-	-	-	-	+
Ambrosia artemisifolia	-	-	-	-	-	-	-	-	-	-	+	-
Erigeron annuus subsp. *annuus*	+	+	+	+	+	+	+	+	+	+	-	+
Erigeron canadensis	-	-	-	-	-	-	-	-	+	+	-	-
Galinsoga parviflora	-	-	-	-	-	-	-	-	+	-	-	-
Matricaria discoidea	-	-	-	-	-	-	+	-	-	+	-	-
Rudbeckia laciniata	-	-	-	-	+	-	-	-	-	-	-	-
Solidago canadensis	-	-	-	-	-	-	-	-	+	-	-	-
Solidago gigantea	-	-	-	-	-	-	-	-	-	-	-	+
Xanthium strumarium	-	-	-	-	-	+	-	-	-	+	-	-

The invasivity and the potential impact of aliens' species on plant diversity from grasslands in the Land of Făgăraș

Among the possible successful causes of the invasion of *Erigeron annuus* populations in grasslands are entomophily pollination, self-, or cross-fertilization, and winged achene dispersed by wind and animals. According to Song et al. (2018), the dominance of *E. annuus* in recently reclaimed areas or disturbed zones is not due to the morphological or biological advantages of this species, such as seed germination, seedling growth, allelopathy, photosynthesis or resistance to disturbance, but to repeated mowing. They stressed the importance of finding the best timing and establishing a frequency of mowing since *E. annuus* begins to bloom earlier than other species. A risk analysis of the invasive potential of allogenic species in the South of Transylvania shows that *E. annuus* has the highest potential for distribution (Zimmermann et al., 2015). The spread of *E. annuus* is mainly due to abandoned farmlands and changes in land use. Proper management and mowing, adapted to land characteristics, can be used to prevent this species from invading the grasslands.

The increase in the distribution area of *Impatiens glandulifera* was attributed to the larger number of seeds produced by a single plant, over 4000, and the specific autochoric spread of the seeds, which reached a distance of 7 m (Helmisaari, 2010). The hydrochory and anthropochory also contributed to the massive presence of the species in the grasslands near the fringe of water courses. It is important to pay attention to the biology of the species and their spring germination.

Other species that prefer wet habitats, such as *Rudbeckia laciniata*, *Oenothera biennis*, and *Echinocystis lobata*, rely on the hydrochory. They enhanced the bank seeds, which became a source for allochthone species in nearby meadows. Monitoring the banks of water courses and occasionally removing patches of vegetation with invasive species could contribute to preventing their spread and allowing native vegetation to regenerate (Jędrzejczak et al., 2022).

Allelopathic effects (Lorenzo et al., 2011) and anthropogenic activity could contribute to the growth of distribution areas for some species such as *Xanthium strumarium, Matricaria discoidea, Galinsoga parviflora, Amaranthus powelii, Ambrosia artemisiifolia, Amorpha fruticosa, Solidago canadensis* or *Erigeron canadensis*. Some of them are related to agricultural land use and crop plants (Figure 5) but they may also extend to grasslands where traditional management is no longer applied.

Figure 5. *Solidago canadensis*, *Xanthium strumarium*, and *Erigeron annuus* in a soibean crop in Lisa area

CONCLUSIONS

Of the sixteen species found around grasslands of the Land of Fagaras, four are found mixed with species from the original vegetal associations.

A significant source of infestation is represented by populations of alien species installed on the watercourse banks.

Besides the morphological and biological characteristics of the species, the invasion success is backed up by the anthropic activity and management of the land.

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FRUCTAN CONTENT AND SOLUBLE SUGARS IN SOME ORNAMENTAL PLANTS

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Abstract

The aim of the current study was to evaluate the content of total fructans, as well as the individual sugars in the different vegetal parts of 13 ornamental plants. Six Allium representatives (Allium sphaerocephalon, Allium aflatunence, Allium 'Gladiator', Allium 'Globus', Allium large-flowered mixed and Nectaroscordum siculum Lindl.), two tulip cultivars, two hyacinth cultivars, Lapiedra martinezii Lag., Tanacetum balsamita and Calendula officinalis were used for the analysis. Ultrasound-assisted water extraction was performed to extract fructans and sugars. Thin layer chromatography and high performance liquid chromatography coupled with refractive index detector (HPLC-RID) method were used for analysis of fructans and sugars analysis. Allium representatives showed the highest content of inulin in their bulb. N. siculum bulb showed the highest fructans and inulin content - 24 g/100 g DW and 17 g/100 g DW, respectively. Fructans in tulip cultivars were in the low values - 3.3-3.8 g/100 g DW. Inulin content in L. martinezii bulbs reached 6 g/100 g DW, while in the roots of C. officinalis - 3.5 g/100 g DW. In the leaves of T. balsamita and L. martinezii only glucose, fructose and sucrose were detected.

Key words: *Allium, fructans, inulin, Lapiedra martinezii, tulip.*

INTRODUCTION

Fructans are reserve carbohydrate in plants. They accumulate in the vacuole, and play an important role in plant vegetation, including osmoregulation, cryoprotection and sink regulation, drought and cold resistance (Yoshida, 2021). Fructans can be found in representatives of several families, such as Asteraceae, Liliaceae, Asparagaceae, Boraginaceae, Campanulaceae, and Triticeae. Many annual and perennial plants accumulate fructans as an energy source that ensures the survival of wintering tissues, and degrade fructans for the sprouting or regeneration of tissues in spring (Hendry and Wallace, 1993). Inulin and fructooligosaccharides (FOS) are part of fructans family widely distributed in varieties of plants as plant storage carbohydrates in medicinal plants, fruits, and vegetables (Van Loo et al., 1995). They are used as dietary fiber or as food ingredients in several food products. The occurrence of fructans (especially in storage organs) is widespread in ornamental geophytes, indicating their importance in this group of plants (Hendry & Wallace, 1993).

Allium species contains carbohydrates, as the most abundant class of natural compounds, as the main representatives includes glucose, fructose and sucrose, together with a series of oligosaccharides and the fructans (Darbyshire and Steer, 1990; Kamenetsky and Rabinowitch, 2010). Most of the studies reported values for fructooligosaccharides and inulin in the bulb of Allium representatives (from 65 to 80% of the dry weight) (Van Loo et al., 1995). Fructans were widespread in the underground part of vegetables (e.g. onion, chicory and asparagus), and ornamentals (e.g., tulip, dahlia and hyacinth) (Ranwala and Miller, 2008).

Interest presents some spices used in culinary practice, as *Nectaroscordum siculum* and *Tanacetum balsamita* L.

Allium bulgaricum (*Nectaroscordum siculum* Lindl., *Nectaroscordum siculum* ssp. *bulgaricum (Janka) Stearn; Allium ursium* var. Dioscoridis) is a plant from Amaryllidaceae family, subfamily Allioideae, Allium species (Popova et al., 2014). In Bulgaria it is known as samardala or 'Bulgarian honey garlic'used in the preparation of spice mixes and salts, and as a seasoning.

Costmary (*Tanacetum balsamita* L.) known also as balsam herb, costmary, sweet tongue and bible leaf is from Asteraceae family and is a traditional medicinal plant of Iranian origin. The plant is cultivated in many countries and it used as an aromatic plant in Europe and Asia. Fresh and dried costmary leaves have a strong lemony-minty flavor and a sweet, astringent taste and are used as flavorings in soups and meats, especially lamb, sausages and cakes (Derakhshani et al, 2011; Gevrenova et al., 2023; Hassanpouraghdam et al., 2022).

Ranwala & Miller (2008) investigated 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection and in 25 they found fructans in the storage organs. In tulip bulbs were detected two reserve polysaccharides - starch (the main reserve carbohydrate) and fructan (a secondary reserve material that exists in vacuoles in a hydrated, colloidal state), and soluble sugars (sucrose, glucose, fructose) (Hobson & Davies, 1978; Kamenetsky et al., 2003). Low-temperature treatment of bulbs is accompanied by degradation of polysaccharides in the bulb scales to lower-molecular-weight sugar molecules (Koksal et al., 2010).

Lapiedra martinezii Lag. (Amaryllidaceae) is an autumn-flowering geophyte that grows in coastal and sublittoral environments. It is endemic in the South-west Mediterranean, mainly distributed in some coastal regions of Spain and North Morocco (Vicedo et al., 2021).

However, until now there may gaps or unsufficinet data in scientific reports about the presence of fructose and sugar content in many ornamental plants.

In this context, the aim of the current study was to evaluate the content of total fructans, as well as the individual sugars in the different vegetal parts of 13 ornamental plants: six Allium representatives (*Allium sphaerocephalon, Allium aflatunence*, *Allium* 'Gladiator', *Allium* 'Globus', *Allium* large-flowered mixed and *Nectaroscordum siculum* Lindl.), two tulip cultivars, two hyacinth cultivars, *Lapiedra martinezii* Lag., *Tanacetum balsamita* and *Calendula officinalis* L. were evaluated as potential sources of inulin and fructooligosacchides.

MATERIALS AND METHODS

The experimental place and period

The bulbs from samardala of the four populations were collected in May 2017 during the flowering stage from their natural habitats in Bulgaria, as follows: Strandzha mountain (coordinates 41°59'53.76"N/27°49'32.56"E), Black Sea coast (42°44'10.02"N/27°39'8.98"E), Eastern Stara Planina mountain (42°45'36.71"N/27°44'54.10"E), and Central Stara Planina mountain (42°45'51.57"N/25°12'16.53"E). Botanical identification was done by botanist Assoc. Prof. Ina Aneva. Bulb from *Allium sphaerocephalon*, *Allium aflatunence*, hyacinth 'Peter Stuyvesant', hyacinth 'Jan Bos', tulip 'Burgundy', tulip 'Aguila' were purchased from Lidle, Bulgaria with the origin of the bulbs the Netherland during January 2021. *Allium* 'Gladiator', *Allium* 'Globus', *Allium* large-flowered mixed were purchased from online magazine during October 2022 www.semenata.bg.

Lapiedra martinezii Lag. was collected in April 2015 from Santa Pola's Cape, Santa Pola (Spain) from 10 m above sea level. Its botanical identification was done by Jorge Juan-Vicedo (co-author in this study).

The roots of *C. officinalis* and fresh leaves of *T. balsamita* were collected from plant garden during September 2021 (Kostievo village, Plovdiv region).

Plant material

L. martinezii Lag. and samardala plant material were lyophylized and finely ground to powder before analysis. Dry samardala leaves were purchased Decrassin Ltd. (Bulgaria). Bulb from other plants were used fresh as they were purchased. The roots of *C.officinalis* and leaves of *T. balsamita* were dried at room temperature and then ground in laboratory homogenizer.

Reagent and chemicals

All reagents were of analytical grade. Nystose, 1-kestose, sucrose, glucose, fructose and resorcinol were purchased from Sigma-Aldrich (Steinheim, Germany).

Moisture content

The moisture content (%) was analyzed on moisture analyzers balance Kern DAB 100-3 (Germany).

Ultrasound-assisted extraction of fructans and soluble sugars from ornamental plants

The dried plants were finely ground in laboratory homeogenizer to the fine powder. The fresh bulbs were cleared and peeled. Then they were homogenized in blender BN1200AL (Gorenje) and used immediately for analysis. The samples were weighted in a centrifuge tube of 50 mL and extracted with distilled H_2O in solid to liquid ratio 1:10 (w/v) for dry samples and 1:4 (w/v) for fresh samples. The extraction conducted in an ultrasonic bath (IsoLab 621.05.001, Germany, ultrasonic frequency 40 kHz and ultrasonic power 60W) for 20 mins, at 75°C. The obtained extracts were filtered, and the residues were extracted once again under the above mentioned conditions. The both extracts were combined and used for further analysis.

Thin layer chromatography (TLC)

TLC analysis was performed to detect the presence of mono-, di-, fructooligosaccharides (FOS) and inulin in water extracts of studied ornamental plants. The TLC plates silica gel 60 F_{254} were used (Merck, Germany) with mobile phase n-BuOH:i-Pro:H_2O:CH_3COOH (7:5:4:2) (v/v/v/v). The TLC plates were dipped for 20 seconds in diphenylamine-aniline-H_3PO_4–acetone and then dried for 5 min at 120°C. The standards glucose, fructose, sucrose, fructo-oligosacchides from agave (Mexico) and inulin from chicory (Raftiline HPX, Beneo, Orafti, Belgium) were used for TLC analysis (Petkova and Denev, 2013).

Spectrophotometric method for analysis of total fructans

The fructans content was determined spectrophotometrically by resorcinol-thiourea reagent as previously described (Petkova and Denev, 2013). The absorbance was measured at 480 nm against a blank sample prepared with distilled water.

High-performance liquid chromatography with refractive index detection (HPLC-RID) analysis of inulin and sugars

HPLC-RID methods were employed for detection of sugars (glucose, fructose, sucrose), 1- kestose, nystose and inulin in ornamental plant extracts. Analysis was performed on HPLC Shimadzu, with LC-20AD pump, analytical column Shodex® Sugar SP0810 with Pb2+ (300 mm × 8.0 mm i.d.), a guard column (50 × 9.2 mm i.d.) at 85°C, refractive index detector Shimadzu RID-10A at 30°C and software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). Distilled water was used as a mobile phase with a flow rate 1.0 mL/min (Petkova et al., 2014).

RESULTS AND DISCUSSIONS

The total fructans, inulin and soluble sugars content in different vegetal part of Bulgarian honey garlic, collected from different parts of the country are summerized Table 1. It was found that in leaves of commercial plant leaves the level of fructans is the lowest one and only 1-kestose was detected. Sucrose and glucose were in the highest values in comparison to their content in bulb of *N. siculum*. It is obviously due to the participation of sucrose in photosynthesis. Fructose was detected as the dominant sugar in samardala leaves - 2.4 g/100 g DW. The highest values of total fructans were detected in samardala bulb, as their content varied between 24.5 to 13.6 g/100 g DW depending from sea level. The highest fructans and inulin content was detected in samples collected from Strandzha mountain and Central Stara Planina mountain. Inulin content in this sample was 24.5-23.5 g/100 g DW, while the lowest levels of it were found in samples collected near to Black Sea coast - 7.4 g/100 g DW. From fructooligosacchrides were detected nystose and 1-kestose, while from sugars sucrose, glucose and fructose were presented. Glucose in the bulb was in the lowest levels - 0.1 g/100 g DW, followed by fructose (0.1-0.4 g/100 g DW). Sucrose varied between 0.3-0.8 g/100 g DW, 1-kestose was in the highest values in samples from the mountain regions, as well as nests. The

highest values of nystose were found in samardala bulb collected form Strandzha mountain - 7.9 g/ 100 g DW.
Additionally, the detailed fructans profile is shown in the samardala bulb (Figure 1, spot 6) and (Figure 3 a). It is obvious that samardala bulbs contain not only inulin and sugars (Figure 3a), but also many fructoolysosacchrides - 1-kestose, nystose, fructofuranosyl-nystose (GF4) and others (Figure 1).

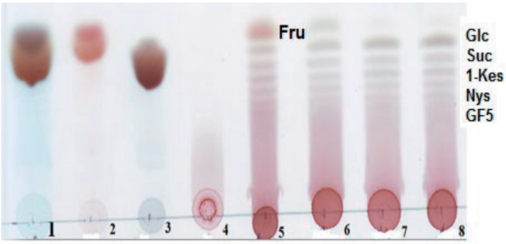

Figure 1. TLC of water extracts from *Allium* sp., where: 1 - glucose; 2 - fructose; 3 - sucrose; 4 - inulin; 5 - fructooligosacchrides from agave, 6 - samardala bulb (*N. siculum*), 7 - *Allium* 'Gladiator' and 8 - *Allium* 'Globus'

This study is the first detailed report about the presence of fructan, fructooligosacchrides and inulin in the bulbs of *N. siculum*. Moreover, the leaves of samardala typically used in culinary as spices showed a similar fraction profile with leaves of *A. schoenoprasum*, as in both 1-kestose were the main representatives of fructooligosacharides (Petkova et al., 2019).
The detail data about the presence of fractions and sugars in different plant organs of 12 ornamental plants are shown in Table 2. The biological material have been presented before. From the obtained results it was found that the bulb of *Allium* 'Gladiator', *Allium* “Globus” and *Allium* large flower mix presents a promising source of total fructan 13-11 g/100 g DW. *Allium sphaerocephalon* and *Allium aflatunence* contain more than 60% less fructan than above mentioned Allium species. The total fructan in them is below 5 g/100 g DW, as inulin content is below 3 g/100 g DW (Table 2). In *Allium* 'Gladiator' and *Allium* 'Globus' were detected inulin, sucrose, glucose, fructose, nystose, 1-kestose and other fructooligosacchrides (Figure 1 spots 7 and 8 and Figure 3). Our observation enriches the information and distribution of fructan and inulin. Fructans were present in some *Allium* species. Ranwala and Miller (2008) found that 25 geophytes investigated possessed concentrations of fructans ranged from 23 to 508 mg g^{-1} DW. From them *Allium* species showed the highest concentration of fructans (about half of the DW). *Allium caeruleum*, *Allium christophii*, *Allium hollandicum* ‘Purple Sensation’, *Allium karataviense*, ‘Ivory Queen, *Allium neapolitanum* and *Allium sphaerocephalon* were investigated and fructan content in them varied from 508-439 mg/g DW (Ranwala and Miller, 2008). In these Allium representatives only sucrose, glucose and fructose were detected. In our research *Allium sphaerocephalon* bulbs contained 4.20 ± 0.07 g/100 g DW fructans which are approximately ten times less than the report of Ranwala and Miller (2008). Similar to their report only sugars were detected, therefore this *Allium* did not so much accumulate fructooligosacchides, but mainly high molecular inulin. *Allium* 'Globus' is a hybrid between *Allium karataviense* and possibly *Allium cristophii* (https://www.pacificbulbsociety.org/pbswiki/index.php/Allium_Globus). *Allium* 'Globus' showed 5 times less fraction content than reported by Ranwala and Miller (2008).
It was found that in costmary leaves were detected only sugars, while in leaves of *L. martinezii* similar to samardala leaves contains except glucose, fructose and sucrose, but also 1-kestose (Table 2). According to Derakhshani et al. (2011), the levels of soluble sugars in leaves of *T. balsamita* reached 136-179 mg/g fresh weight. In our study, sugar content in *Tanacetum balsamita* was 1.48 g/ 100 g DW. Marigold (*C. officinalis*) roots and bulbs of *L. martinezii* contained inulin, nystose, 1-kestose, sucrose, glucose and fructose. This is the first report about presence of inulin and sugars in this both plants. Inulin content in bulb of *Lapiedra martinezii* Lag. was 6.82 ± 0.11 g/100 g DW, while its content in marigold roots was twice lower - 3.51±0.12 g/100 g DW. Bulbs of tulip ('Burgundy' and 'Aguila') and hyacinths ('Jan Bos' and 'Peter Stuyvesant') were evaluated for fructans and sugars content. It was found that tulip bulb contains mainly sugars and small amout of inulin (below 3 g/100 g DW) (Table 2). The detailed profile of fructans and soluble sugars content in bulb of different tulip and hyacinth representatives was shown

(Figure 2 and Figure 3). According to Parkin (1899) the inulin in the hyacinth is very similar to that of the garlic. The highest inulin content was found in hyacinth 'Peter Stuyvesant' - 4.00 g/100 g DW, while total fructans in its bulb was 6.04 g/100 g DW. It was reported that in bulbs of narcissus (*Narcissus hybrids* Hort), onion (*Allium victorialis* L.) fructan content reached 19.8%, while in lily, onion, tulip, hyacinth, camash (wild hyacinth) (*Camassia quamash*) was in the range 12-22% (Bagaoutdinova et al., 2001).

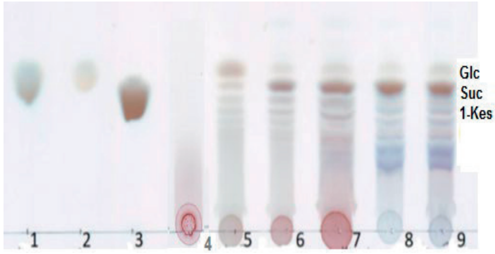

Figure 2. TLC of water extracts from the bulbs of ornamental plants, where: 1 - glucose; 2 - fructose; 3 - sucrose; 4 - inulin; 5 - fructooligosacchrides from agave; 6 - Hyacinth 'Peter Stuyvesant'; 7 - Hyacinth 'Jan Bos'; 8 - tulip 'Burgundy'; 9 - tulip 'Aguila'

Table 1. Quantity of sugars and fructans in Bulgarian honey garlic or samardala (*Nectaroscordum siculum* Lindl.), g/100 g DW

Samples	Location	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
Samardala leaves	Commercial	4.5±0.1	n.d.	n.d.	0.7±0.1	0.9±0.2	0.8±0.2	2.4±0.2
Samardala bulb 1	Strandzha mountain	24.5±0.2	14.5±0.3	7.9±0.3	1.0±0.1	0.8±0.3	0.1±0.1	0.2±0.1
Samardala bulb 2	Black Sea coast	13.6±0.1	7.4±0.2	1.9±0.3	0.5±0.1	0.3±0.1	0.1±0.1	0.4±0.1
Samardala bulb 3	Eastern Stara Planina mountain	20.1±0.2	12.6±0.4	5.1±0.3	1.0±0.2	0.6±0.1	traces	0.3±0.1
Samardala bulb 4	Central Stara Planina mountain	23.5±0.3	17.1±0.1	4.0±0.3	0.8±0.1	0.5±0.1	traces	0.2±0.1

n.d. = not detectable. Values are means ± SD, n=3

Table 2. Fructans and sugars content in different ornamental plants, g/100 g DW

Plant	Family	Plant organs	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
Allium sphaerocephalon	Amaryllidaceae	bulb	4.20±0.07	1.95±0.01	n.d.	n.d.	0.18±0.05	0.10±0.02	0.56±0.02
Allium aflatunence	Amaryllidaceae	bulb	4.78±0.17	2.51±0.01	n.d.	0.11±0.01	0.08±0.03	n.d.	0.51±0.06
Allium 'Gladiator'	Amaryllidaceae	bulb	11.21±1.07	7.42±0.05	0.05±0.01	0.12±0.02	0.21±0.05	0.53±0.06	2.23±0.11
Allium 'Globus'	Amaryllidaceae	bulb	10.17±1.417	7.21±0.05	0.02±0.01	0.92±0.02	1.01±0.02	1.65±0.03	2.44±0.08
Allium large flower mix	Amaryllidaceae	bulb	12.42±1.14	6.21±0.12	1.03±0.02	0.92±0.03	1.72±0.02	0.62±0.04	1.52±0.06
Tanacetum balsamita	Asteraceae	leaves	0.47±0.15	n.d.	n.d.	n.d.	1.28±0.12	0.16±0.03	0.04±0.01
Calendula officinalis L.	Asteraceae	roots	9.71±0.45	3.51±0.12	0.51±0.03	0.32±0.02	2.23±0.05	1.21±0.04	2.25±0.12
Lapiedra martinezii Lag.	Amaryllidaceae	bulb	7.84±0.15	6.82±0.11	2.63±0.02	2.72±0.06	4.55±0.02	0.44±0.06	2.43±0.05
		leaves	n.d.	n.d.	n.d.	2.12±0.02	2.96±0.05	2.14±0.07	4.22±0.02
Tulip 'Burgundy'	Liliaceae	bulb	3.39±0.15	1.54±0.12	0.51±0.02	n.d.	2.14±0.05	0.14±0.02	n.d
Tulip 'Aguila'	Liliaceae	bulb	3.89±0.21	2.72±0.09	n.d.	n.d.	3.03±0.02	0.32±0.01	0.11±0.02
Hyacinth 'Jan Bos'	Asparagaceae	bulb	3.73±0.18	2.42±0.11	0.41±0.11	0.22±0.11	0.63±0.11	n.d.	n.d.
Hyacinth 'Peter Stuyvesant'	Asparagaceae	bulb	6.04±0.34	4.00±0.12	n.d.	0.22±0.11	0.32±0.11	0.71±0.11	0.11±0.11

n.d. = not detectable, values are means ± SD, n=3

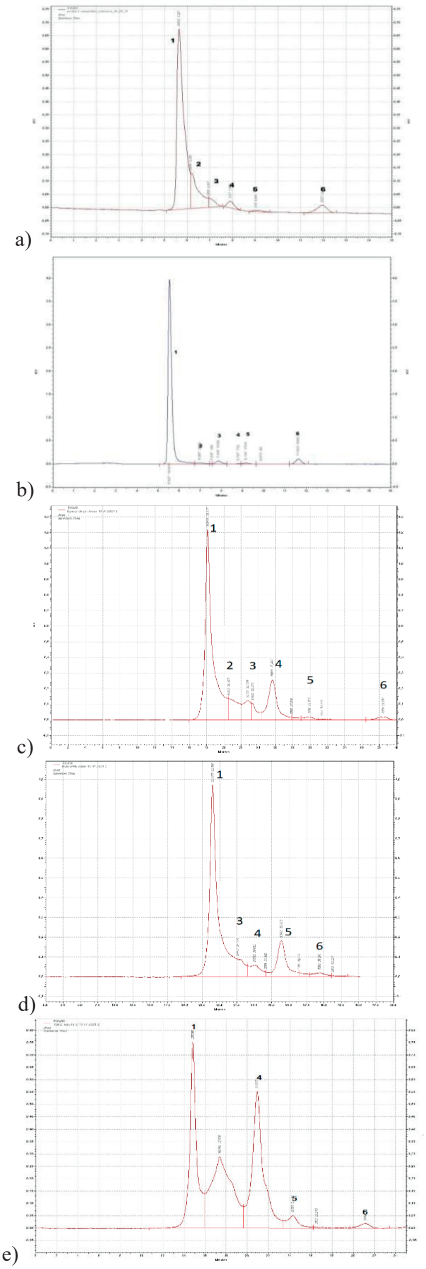

Figure 3. HPLC chromatograms of water extracts from different ornamental plants, as follows: a) samardala (*Nectaroscordum siculum* Lindl.) - bulb 1; b) Allium 'Globus'; c) hyacinth 'Jan Bos'; d) hyacinth 'Peter Stuyvesant'; e) tulip 'Aguila', where: 1 - inulin, 2 - Nystose, 3 - 1-kestose, 4 - Sucrose, 5 - glucose and 6 - fructose

Fructans content in *Hyacinthus orientalis* (‘Carnegie and ‘Pink Pearl’) reached 284 mg/g DW (Ranwala and Miller, 2008). In our studies fructans in bulb of hyacinth 'Jan Bos' and 'Peter Stuyvesant' was 3.73 and 6.04 g/100 g DW. Our data for fructans in hyacinth bulb were lower than reports of some authors (Bagaoutdinova et al., 2001; Ranwala and Miller, 2008), but content of glucose and sucrose were comparable with the study of Ranwala and Miller (2008). However, Enciu et al. (2021) detected only glucose and fructose in the bulbs of hyacinth 'Jan Bos' between 24 and 26%. However, fructans content in tulip bulbs was comparable to reported values for fructans in *Tulipa gesneriana* ‘Apeldoorn’ and ‘Monte Carlo’, *Tulipa tarda* and *Tulipa turkestanica* - 23-54 mg/g DW (Ranwala and Miller, 2008). The lowest inulin and fructans content were detected in bulb of tulip 'Burgundy' - 1.54 and 3.39 g/100 g DW, respectively.

The differences in fructan and inulin values in the bulb of *Allium* species, tulip and hyacinth could be explained by environmental and storage conditions, age of the plant, and time of harvest. In case of hyacinth bulb, their exposure to low temperatures leads to increased glucose, fructose and sugar levels (Koksal, 2010). Onion bulbs cv ‘Hyduro’ decrease fructans content during storage as form 3 weeks fructans content from 39.1 g/100 g DM can decrease to 16 g/100 g DM after storage period 40 weeks (Hansen, 1999).

CONCLUSIONS

In the current study thirteen ornamental plants were investigated for fructans and sugar content. It was found that the leaves of *Tanacetum balsamita* and *Lapiedra martinezii* contains only sugars, as glucose, fructose and sucrose. However, bulbs and roots of other investigated representatives, except sugars contains fructooligosacchides and inulin, as reserve polysaccharides. The highest inulin content was found in bulb of samardala, followed by *Allium* 'Gladiator', *Allium* 'Globus' and *L. martinezii* bulbs. To the best of our knowledge this is the first report for evaluation of carbohydrates profile and fructans content in *N. siculum*, *T. balsamita* and some *Allium* representatives. The present research enrich the information about fructans and inulin content in hyacinth, tulip and some other *Allium* species and reveals the potential of bulbs of samardala, *L. martinezii* and some *Allium* species as a potential source of fructans.

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THE GROWTH DYNAMICS OF PAULOWNIA TREES CULTIVATED AS ENERGY PLANTATIONS IN THE FOREST-STEPPE ZONE OF UKRAINE

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Abstract

The main objective of this study was to establish the impact of varietal characteristics of Paulownia trees on growth and productivity depending on the density of planting in the forest-steppe zone of Ukraine. The greatest increasing of Paulownia Clone 112 trees height - 425 cm was fixed in the first year of vegetation at a density of 625 plants/ha. The same tendency observed in the fourth year. The highest height of Paulownia Clone 112 during the vegetation season was observed in June (170 cm), Paulownia tomentosa - 140 cm. The smallest increase in height was achieved in October, 40 cm and 35 cm, and in April, 15 cm and 10 cm, respectively. A first year trunk of a Paulownia tree reaches a diameter of 4-6 cm, a second year trunk - 7-10 cm, a three-year trunk - 11-16 cm, a four-year trunk - 15-23 cm. A linear relationship between the height and diameter of Paulownia trees was established. It is enough to measure one leaf dimension (length or width) to determine the area of the leaf surface.

***Key words**: Paulownia, variety, clone, growth parameters, density of plantations*.

INTRODUCTION

An increase in the concentration of carbon dioxide and greenhouse gases in the earth's atmosphere is one of the main causes of climate change on earth (Sinchenko, 2020). These problems lead to the search for new ways of management and effective technologies. Further use of forest resources and existing wood potential in Ukraine is unacceptable, as the average afforestation of the territory of Ukraine is 16.5% (Shvidenko et al., 2017). This is one of the lowest indicators among European countries. Bigger environmental safety from burning biomass is the main advantage compared to fossil fuels (Kraszkiewicz et al., 2020). During the combustion of biofuels based on plant biomass, significantly less sulfur oxide is formed compared to fossil fuels.

Energy plantations based on tree crops in a minimal period provide an opportunity to obtain a significant volume of high-quality wood products. It is necessary to use mainly fast-growing trees to create energy plantations with a short vegetation period 3-5 years (Wang & Shogren, 1992). One of the promising, economically and ecologically justified crops is Paulownia, which is undemanding in maintenance, investments are repaid quite quickly due to intensive growth rates and the volume of final products (Beel, 2005). Planting paulownia is able to restore in the shortest possible time areas of land affected by fire, landslides, flood scour, or other land scarification (Innes et al., 2009). Paulownia trees have shown a high growth rate during the first three years after coppicing (Criscuoli et al., 2022). Growth rate sharply decreased starting from the fourth year because of the need for greater planting spacing. Paulownia trees cultivation can either increase or decrease crop production, depending on the manipulation of the tree density and rotation length (Yin & He, 1997). Economic profits of timber-oriented Paulownia stands with intercrops are greater than those of crop fields with scattered Paulownia trees. The combined net returns of crops and trees are generally higher than those of the control, ranging from 50% to 100% (Jiang et al., 1994). Paulownia tree can develop deep root system (up to 8 m) depending on soil structure (Szabo et al., 2022). Paulownia at least does not consume substantially higher amounts of water than a few other tree species that are commonly utilized in plantations settled in arid and semi-arid conditions (Baier et al., 2021). However, it is necessary to consume up to 2000 litters of water per tree to reach a production of 4.3 t/ha during the first cut (García-Morote et al., 2014). There were shown differences in the Paulownia trees clones growth dynamics regarding to local environmental and climatic conditions (Jakubowski, 2022). Dry biomass production yields in the second year of cultivation range from 1.5 t/ha to 14 t/ha. Areas abandoned for agricultural use and other categories of land that are not suitable for use (low-productivity, waterlogged, eroded, etc.) can serve as energy plantations in the best way (Blanco-Canqui, 2016; Icka et al., 2016). This will also make it possible to significantly increase the efficiency of the use of such areas, significantly improve the ecological state of the environment and create favorable conditions to cultivate energy plantations (Flynn & Holder, 2001). Significant increase in microbial activity of Cambisol was fixed after one year of tree planting (Wozniak et al., 2022). It was proved the prospects of the Paulownia as fast-growing plant for reclamation on abandoned mineland (Fokina et al., 2020). An unusual weather event (freezing temperatures, drought and strong winds) can be the reason of weak performance of Paulownia plantations during the early years of trees growth (Olave et al., 2015; Pástor et al., 2022). Five years after planting only 25% of planted individuals of Paulownia showed signs of crown damage caused by wind (Barton et al., 2007). The rest individuals of *P. cotevisa* in this study were not affected by any other negative environmental factor or biological pest. The clone *Paulownia elongata* x *fortunei in vitro* 112 was selected in Spain to resist temperature extremes (from −25 to +45°C) and to grow at a faster rate compared to other Paulownia species and hybrids (Ayan et al., 2006). It was established that the age of 5 years is the minimum cutting age for the use of this wood for solid fuel, such as pellets, and as solid wood (Esteves et al., 2022). The main prospects of growing Paulownia trees in short cycles is to produce woody biomass for bio-goods (Kalaycioglu et al., 2005; Caparro et al., 2008; Schroder et al., 2018). The fast growing Paulownia trees can serve also for such purposes as mulching and leafy biomass for fodder (Stewart et al., 2018). Leaves of Paulownia have the same as alfalfa nutritious values including fats, sugars and proteins for cattle nourishment to be are suitable as forage used for ruminants, non-ruminants animals and poultry feeding (Alagawany et al., 2022). The β-carotene content in the Paulownia leaves rich 7716 μg/g (Steier et al., 2022).

The use of residues generated in the forest biomass industry (pine bark and biomass ash) as a soil amendments had a positive effect on the soil microbial activity (Moreno et al., 2017). Extending the length of the fertilization period to 10 years in a Paulownia plantation can be an effective management practice and tool to provide efficient carbon sequestration in the soil carbon pool and for rational application of organic fertilizer in Paulownia plantations (Wu et al., 2022). An significant positive effect of the slurry application in plantation of Paulownia was established in the diameter at breast height and total stand height (Menino et al., 2022).

The main objective of this study was to establish the impact of varietal characteristics of Paulownia trees on growth and productivity depending on the density of planting in the forest-steppe zone of Ukraine.

MATERIALS AND METHODS

The research was carried out during 2018-2021 at the field experimental station of the Institute of Bioenergy Crops and Sugar Beet of the Ukrainian National Academy of Sciences. The analysis of the dynamics of the weather conditions during the season of vegetation was made for 2018-2021 (Figure 1).

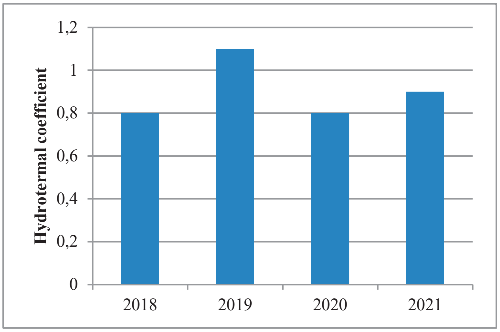

Figure 1. Hydrothermal coefficient for 4 years

According to the analysis of changes in the hydrothermal coefficient (HTC), the conditions for growing paulownia in the forest-steppe zone of Ukraine are optimal.
The soil of the experimental field - sod - podzolic sandy loam had the following parameters of the topsoil: pH - 5.3-5.5, total humus content 0.50-0.62%; mobile phosphorus and potassium - 160-180 and 50-65 mg/kg of soil, respectively; hydrolyzed nitrogen according to Kornfield - 39-45 mg/kg of soil. In general, the soil has low fertility.
Paulownia plantations were established according to tree planting scheme 4 x 4 m with a density: A - 500, B - 625, C - 833 and D - 1050 pcs/ha.
Deep plowing of the soil was done in autumn along with the introduction of herbicides. Soil cultivation was done in the spring. Holes for planting seedlings were made with a motor drill with a diameter of 60 cm and a depth of 40 cm. Mineral and organic fertilizers were applied before the same time. Seedlings grown in vitro were subjected to a two-week adaptation to natural lighting and daily temperature changes before planting in open ground to a permanent place. The tested cultivars were Paulownia Clone - 112 and Paulownia Tomentosa P. Annual growth, trunk diameter, and leaf surface area were determined annually (Figures 2 and 3).

Figure 2. Measurement of Paulownia plants height

It was necessary to make a technical cut after the first year of vegetation at a height of 2-3 cm from the ground and remove young, newly formed shoots (stepchildren). The diameter of the trunk was measured at a height of 1 m.

Figure 3. Measurement of Paulownia plants leaf area

The height and number of leaves on the selected plants in the research plot of paulownia were measured. Then the plants were cut and the total leaf surface area and the mass of each stem with leaves were determined. The research was intended to establish the stochastic dependence of the leaf surface area of paulownia plants on its morphometric indices. The circle modeling was used for this. The correlation and regression analyses of the dependence of leaf surface area on leaf size were carried out using the "Pitiole" software application.

RESULTS AND DISCUSSIONS

The density of plants is related to the area of their nutrition. The greatest of Paulownia Clone 112 trees height - 425 cm was fixed in the first year of vegetation at a density of 625 plants/ha. The same tendency observed in the fourth year (Figure 4). The similar results were obtained in other case study when growth indices after the third year were significantly reduced for plants located in the interior portion of the stands because of the competition between trees (Criscuoli et al., 2022). The need for a larger planting spacing confirmed after the third year.
The greatest development of Paulownia Clone 112 during the vegetation season was observed in June (170 cm), *Paulownia tomentosa* - 140

cm. The smallest increase in height was achieved in October, 40 cm and 35 cm, and in April, 15 cm and 10 cm, respectively (Figure 5). A first year trunk of a Paulownia tree reaches a diameter of 4-6 cm, a second year trunk - 7-10 cm, a third year trunk - 11-16 cm, a fourth year trunk - 15-23 cm (Figure 6).

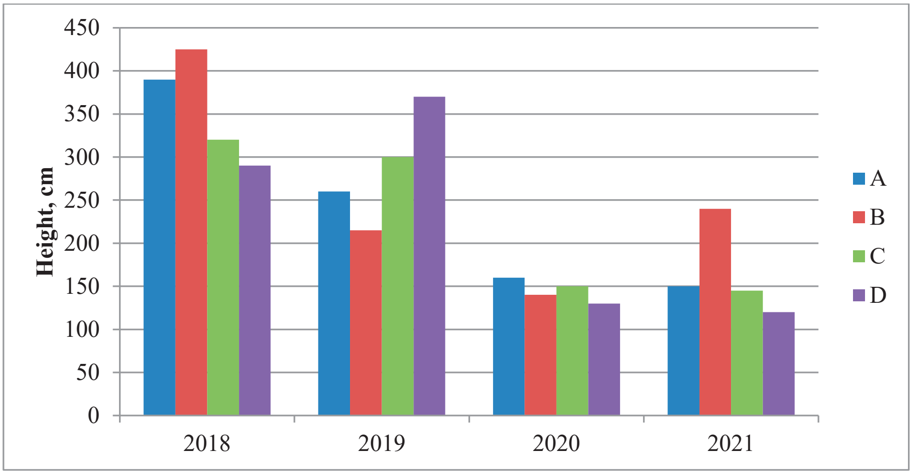

Figure 4. The growth dynamics of Paulownia Clone 112 trees for 4 years, cm

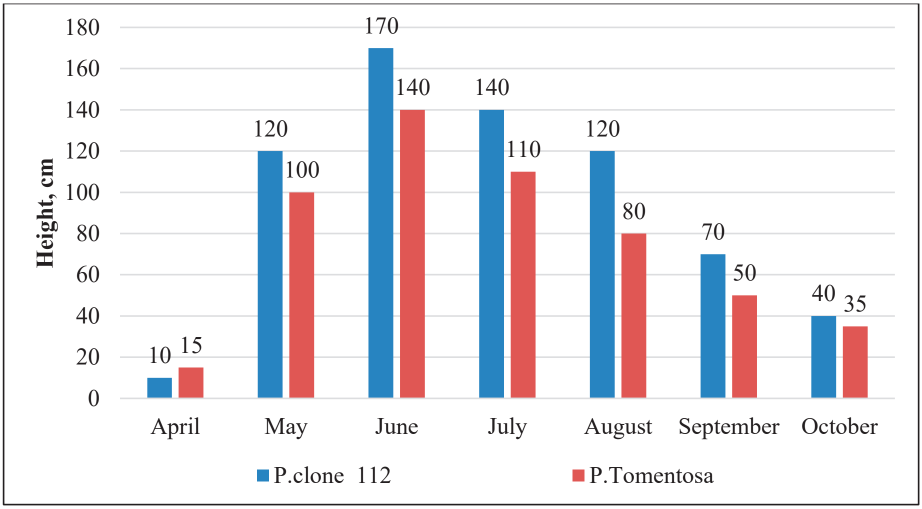

Figure 5. Average monthly growth of Paulownia Clone 112 and *Paulownia tomentosa* by month

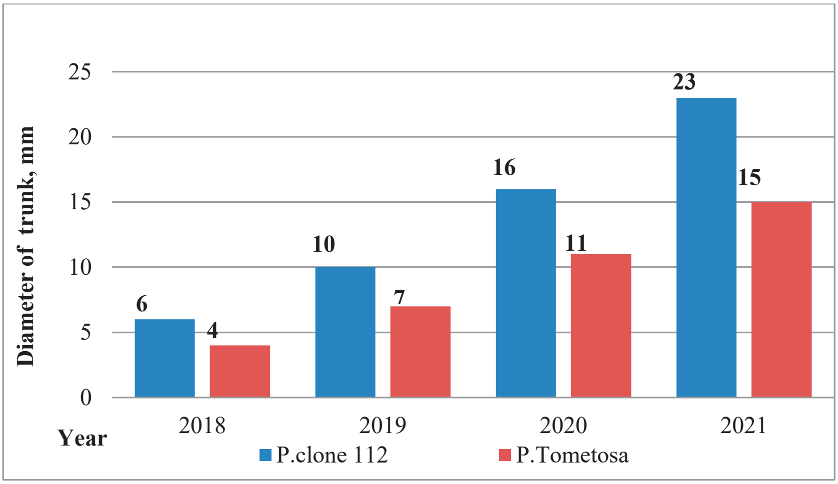

Figure 6. The average diameter of the trunk of Paulownia Clone 112 and *Paulownia tomentosa* by years of vegetation

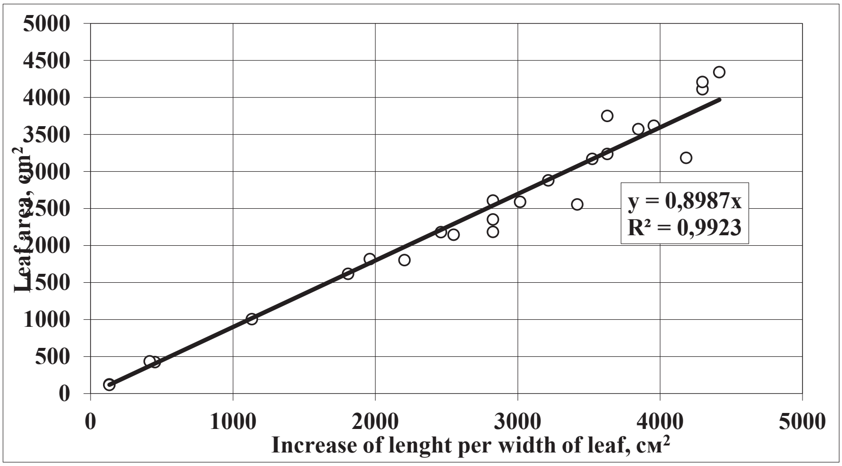

Figure 7. The average diameter of the trunk of Paulownia Clone 112 and *Paulownia tomentosa* by years of vegetation

The results of determining the dependence of the leaf surface area of paulownia plants on the increase of the length of the leaf and its width are shown in the Figure 7. It is enough to measure one leaf dimension (length or width) to determine the area of the leaf surface.

CONCLUSIONS

The greatest increasing of Paulownia Clone 112 trees height - 425 cm was fixed in the first year of vegetation at a density of 625 plants/ha. The same tendency observed in the fourth year. A first year trunk of a Paulownia tree reaches a diameter of 4-6 cm, a second year trunk - 7-10 cm, a third year trunk - 11-16 cm, a fourth year trunk - 15-23 cm. The growth of leaves was especially large in the first and second year of plant vegetation.

Based on the results of research, it was established that the dynamics of the number of trees per hectare, with a square layout of 4x4 m, should be 625 trees per hectare.

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LANDSCAPE DESIGN PROCESS OF A PRIVATE EVENTS VENUE GARDEN IN IASI COUNTY, ROMANIA

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Abstract

Due to urban acceleration and current social and economic development, gardens have begun to receive increased attention from society. Just as therapeutic gardens can be designed for hospitals or green relaxation spaces for employees, gardens can also be designed for event locations. Private event venues have begun to pay more attention to green space around buildings to provide attendees with green areas to relax and socialize. More and more event locations are located on the city's outskirts, thus offering the opportunity to create perspectives with natural views. In the present work, it was desired to design a private garden for events with multiple purposes (civil weddings, christening parties, marriage parties, corporate parties, etc.), in the Iasi city, Romania, on the shores of Lake Ciric II. The design style adopted had sinuous forms and minimal intervention on the existing tree vegetation. The proposed vegetation was represented by deciduous trees that will provide decoration through shape and color and by shrubs grouped in compositions that will provide decoration in all seasons.

***Key words**: landscape design, private garden, multifunctional garden, design process, events venue.*

INTRODUCTION

Landscape design is a multifaceted field that involves the artful composition of hardscape and softscape elements to create aesthetically pleasing and functional outdoor spaces.

According to Hansen (2010), problem-solving through horticultural science and spatial organization is at the core of *landscape design*. The goal is to create outdoor "rooms" that can be utilized for various purposes.

Beaulieu (2020) describes *landscape design* as an art that involves arranging the features of an area of land with both aesthetic and practical considerations in mind. Hardscape elements like pavers and softscape elements like plants are essential components often used to achieve the desired effect. This approach enables the designer to create visually stunning outdoor spaces that are functional.

Eckbo and Clifford (2019) note that *landscape design* is vital in enhancing the settings of buildings, public areas, recreational areas, and parks. It is a decorative art closely related to architecture, city planning, and horticulture. By incorporating various design principles and scientific knowledge, landscape designers can create outdoor spaces that are visually appealing and serve practical purposes.

Overall, *landscape design* is a complex field that involves the integration of multiple disciplines to create outdoor spaces that are both aesthetically pleasing and functional. Through the use of hardscape and softscape elements, designers can create outdoor spaces that are both beautiful and practical, enhancing the overall setting of the built environment.

Rapid urbanization can lead to many problems, including air, water, and soil pollution, social and economic problems, and loss of green spaces (Zhang et al., 2023; Wang et al., 2021). One way to solve these problems is by creating green spaces through landscape design to meet the needs of urban amenities and quality of life (Mouratidis, 2021; Hangan et al., 2018; Grecu et al., 2018).

Nowadays, the different forms of urban green areas represent one of the essential elements of the built environment, directly affecting physical activity, health, and mortality (Dascalu et al., 2018; Grecu et al., 2018; Vujčić Trkulja and Tomićević, 2018). A form of urban green space is a forest near or in a city. These forests (called urban forests) provide citizens various economic, ecological, environmental, and social benefits (Lee and Kim, 2022; Gao, 2018).

Event companies give special attention to these urban forests because they offer many advantages: natural aesthetics for a ceremony and reception, breathtaking views, and ever-present vegetation in the wedding season gives the wedding a much more relaxed feel than a traditional ballroom.

The wedding celebration has many traditions and customs. In Romania, traditional weddings are a world in themselves: they start early in the morning and sometimes end the following day, passing through several essential stages and taking the family to different places. Modern weddings go for nature, relaxed ceremonies, and break out of traditional patterns. Garden ceremonies ideally suit couples with a great love for the outdoors and endless styling opportunities for a garden wedding (Boangiu, 2021; Yarushina, 2021).

Gardens at event venues can also be designed with multiple functions, just as therapeutic gardens can be created for hospitals or corporate green spaces can be designed for employee relations purposes (Campbell et al., 2019; Jiang et al., 2018; Naderi and Shin, 2008).

This paper presented a case study of the landscape design process of a private garden for events with multiple purposes (civil weddings, christening parties, marriage parties, corporate parties, etc.), in Iasi city, Romania, on the shores of Lake Ciric II.

MATERIALS AND METHODS

The targeted site has an area of 4516 m^2 and is located in Iasi city, Romania, on the shore of Lake Ciric II, with coordinates 47°11'05.0"N 27°36'03.1"E (Figure 1).

The Ciric Park Forest is located in the northeastern part of the city at a distance of 6 km from the city center. Covering an area of 252 hectares, the Ciric forest, whose planting began in 1936 and was completed in 1963, consists of native and exotic trees and includes tourist cabins, cottages, restaurants, access roads on both sides of the lakes, and a camp for children. Being a popular leisure place for the inhabitants of Iasi, this area was rehabilitated in 2011-2014 in order to increase its attractiveness. The surrounding forest offers a perfect setting for activities such as paintball, tree climbing (adventure park), or wall climbing. The range of restaurants and the number of people spending summer weekends here has grown (Humelnicu, 2022; Nicoara et al., 2009).

Figure 1.Project site location(Google Maps, 2023)

The climate in the Iasi County area has a pronounced temperate-continental character, integrating organically with the natural conditions of Moldavia. The range of temperatures ranges from the absolute maximum (40°C) in July to the absolute minimum (-30°C) in January. The multiannual average temperature for the Iasi area is 10.6°C, minimum of 8.1°C in January maximum of 28.4°C in July. The average annual precipitation in the Iasi agricultural ecosystem is 529 mm (Spatareanu et al., 2018).

The list of plants (Table 1) was carefully chosen according to their ecological requirements (light, water, soil), the way they are combined, the decorative elements (habit, shape, color of leaves, flowers, and fruit), and the possibility of decorating over a more extended period because a garden must have a decorative effect all year round.

As this type of garden is aimed at the private sector, other aspects such as the concept and theme of the location, the type of customers, and the types of events to be held in the location will be taken into account.

The design process of the landscape project was based on the following steps: inventory and survey of the site and its immediate surroundings; analysis and evaluation; concept and design; control, revision, and approval; final design project (Dinç and Gül, 2022; Design Commission for Wales, 2016; Turner, 2014; Booth and Hiss, 2011; Filor, 1994). All design principles were considered (Booth and Hiss, 2011; Hansen, 2010).

The design programs used in the project were AutoCAD 2D for sketching and technical plans, SketchUp for 3D modeling, Lumion for rendering, and Photoshop for image post-processing.

Table 1. List of proposed trees and shrubs

	Species name	QTY
Trees	*Prunus cerasifera* `Nigra`	4
	Rhus typhina `Dissecta`	5
	Salix matsudana `Tortuosa`	6
Shrubs	*Berberis thunbergii* `Atropurpurea`	34
	Buddleja davidii `Purple Prince`	8
	Buddleja davidii `White Ball`	6
	Chamaecyparis pisifera `Baby Blue`	10
	Cornus alba `Argenteomarginata`	41
	Cotinus coggygria `Royal Purple`	16
	Cotoneaster horizontalis	32
	Hibiscus syriacus `Ardens`	7
	Hibiscus syriacus `Russian Violet`	5
	Juniperus communis `Arnold`	34
	Juniperus horizontalis `Glauca`	41
	Juniperus sabina `Tamariscifolia`	19
	Juniperus scopulorum `Blue Arrow`	32
	Juniperus squamata `Blue Compact`	15
	Penisetum alopecuroides `Little Honey`	34
	Perovskia atriplicifolia `Russian Sage`	49
	Picea pungens `Glauca Globosa`	6
	Pinus mugo `Mops`	63
	Prunus laurocerasus - hedge	147
	Syringa vulgaris `Kathrine Havemeyer`	14
	Tamarix ramosissima `Rubra`	13
	Viburnum opulus `Roseum`	18

RESULTS AND DISCUSSIONS

Inventory and survey of the site and its immediate surroundings

In light of the intended purpose of the landscape design, which is to serve as a venue for various social events such as civil weddings, christening parties, wedding parties, and corporate events, the landscape architect established a set of objectives. These objectives include the development of an impressive main entrance, the creation of a thematic lounge area with dining facilities, the establishment of two areas specifically designed for civil weddings, the utilization of the gazebo and lighting poles from the previous location, the formation of plant compositions, and the establishment of lawn areas.

The site's vegetation comprises mature willow, black locust, beech, linden trees, dogwood hedges, and a limited number of rose and common yew bushes. A comprehensive inventory and survey were conducted to comprehend the site’s limitations better, and the site's problem areas were identified. Figure 2 illustrates the site's relatively problematic areas considered during the design process.

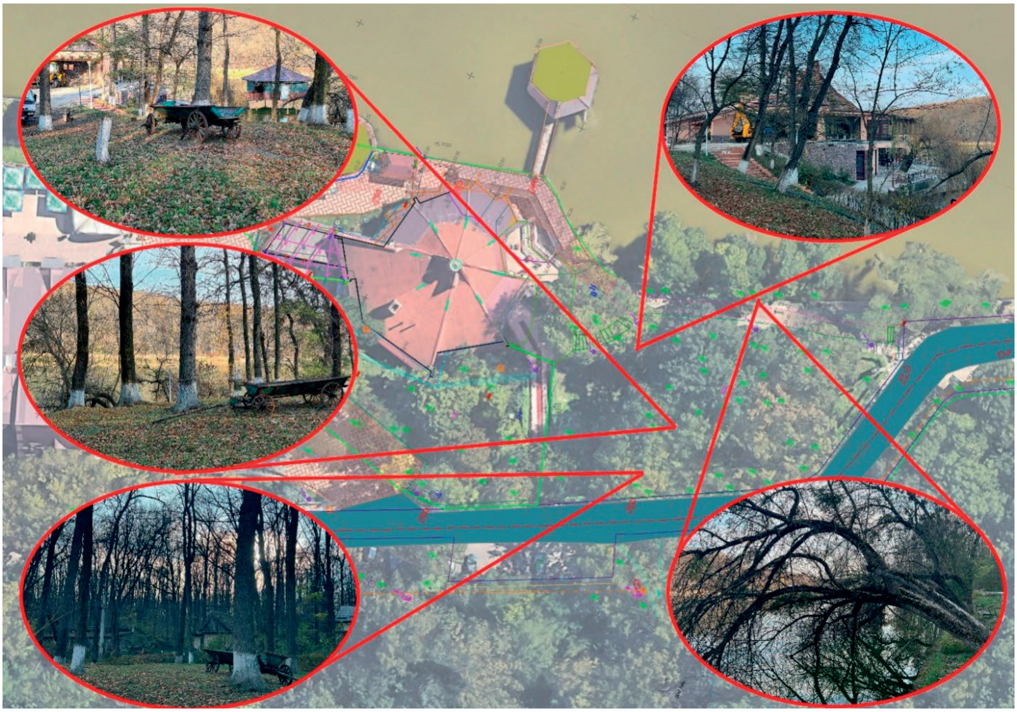

Figure 2. Inventory and survey of the site

An inventory of 66 trees has been conducted, revealing that the majority are mature and healthy with good growth patterns, providing ample shaded areas throughout the site. Nonetheless, a few older trees are at risk of collapsing due to rot or possess unsightly appearances. As a result, it has been determined that these problematic trees will need to be removed and replaced with either the same or a decorative species of tree, where feasible. This will mitigate safety concerns and maintain the site's aesthetic appeal.

Analysis and evaluation

According to the purpose and objectives of the development, the functional diagram in Figure 3 was proposed.

a. Areas for civil weddings will be created on the existing pontoon and plateau within the site (Figure 3a).

b. The themed lounge area will be created close to the building to facilitate access for attendees and employees (Figure 3b).

c. Social areas will be created on the lakeshore to take advantage of the natural scenery on the opposite shore. Another social space will be built next to the primary access (Figure 3c).

d. The primary access will be created perpendicular to the building entrance. It will be provided with two alveoli at the property and building entrances (Figure 3d).

e. Lawn areas will be proposed in the regions intended to be frequented to provide an aesthetically pleasing and manicured appearance (Figure 3e).

f. The storage and employee access area will be screened.

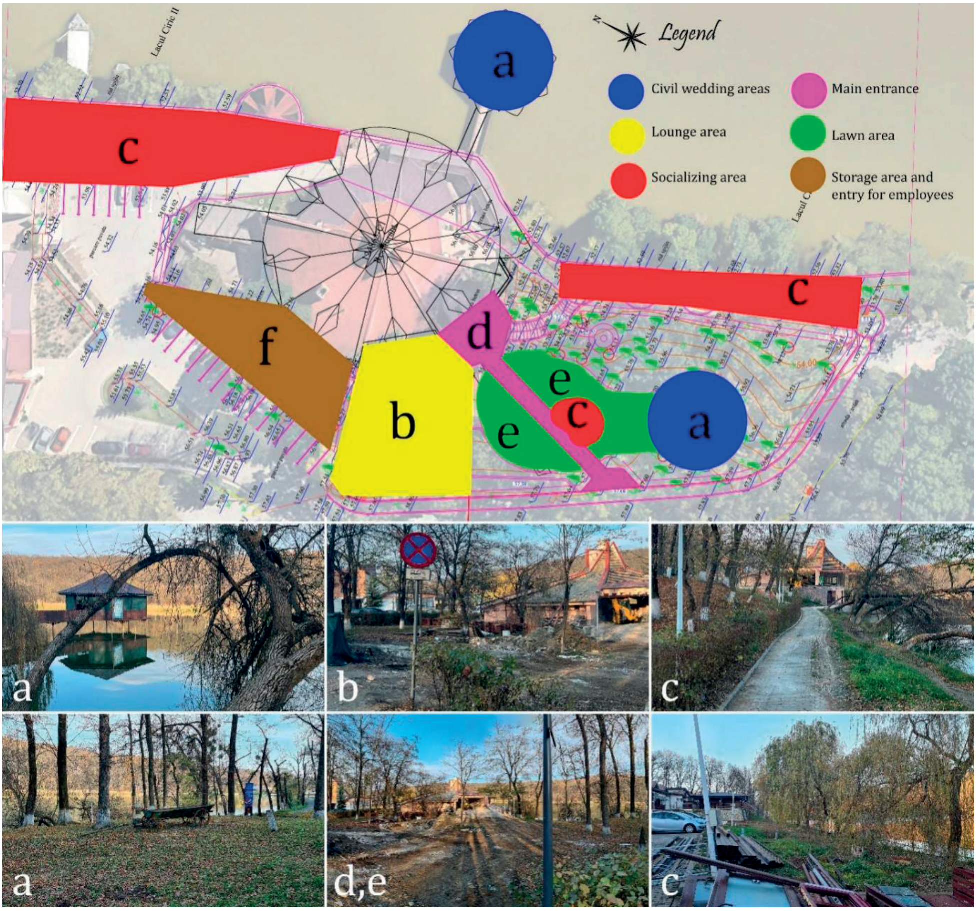

Figure 3.Documentation of the existing situation and proposed functional diagram

The new design concept of these buildings foresees sinuous shapes that recreate the contours of lotus flowers. An architectural firm will rehabilitate and revitalize the building and the pontoon. Two ballrooms are planned in the main building. On the ground floor, there will be the main ballroom with a capacity of 300 people, and on the basement, there will be a hall with half the capacity of the previous ballroom.

Concept and design

Previous research on the effectiveness of specific design elements in creating a relaxing and visually attractive outdoor space informed the design of the private events venue garden. A study by Deng et al., 2020 found that certain landscape types, elements, and components in urban parks can promote physiological and psychological restoration. These include the use of natural elements such as trees and water, as well as the incorporation of design elements such as winding paths and curved shapes. The study also found that the presence of various functional areas within the park, such as areas for relaxation and physical activity, can contribute to a positive overall experience for visitors. By incorporating these elements into the design of the private events venue garden, the aim is to create a space that promotes restoration and relaxation for attendees.

Figure 4. Main entrance preliminary sketch

Based on the proposed functional diagram and owners' requirements, the first idea of the main entrance was sketched “freehanded” using Photoshop (Figure 4). The main entrance is highlighted by placing a red carpet and highlighting the route to the building with arches. These arches will also provide light during the night. A winding trail to the other proposed areas is also sketched.

Figure 5. Preliminary concept and design - plan view (original)

The initial concept for the layout involved incurporating the sinuous contours of the building into the green space design, resulting in a cohesive and harmonious arrangement (Figure 5).

Figure 6 provides visual representations of all the preliminary design renderings.

An area for civil weddings has been created on the existing plateau area. This area is proposed to be leveled to accommodate the gazebo from the previous location. This leveling also facilitates individual seating areas to allow attendees to view the civil ceremony.

Figure 6. Preliminary design renderings

The proposed lounge area consists of two sub-areas. The lounge sub-area close to the building benefits from an outdoor bar. Considering the modern style of restructuring of the building, this lounge area is composed of thematic modules following the four elements of nature: ground, air, water, and fire. The ground-level LED strips in specific colors highlight these elements. The lounge sub-area close to the property boundary is formed into two platforms. A smaller, round-shaped platform serves as a podium space for small wedding parties or as a stage for outdoor parties. The purpose of the second platform is to provide space for tables or as a dance floor.

Three main areas of socialization have been proposed, even if the whole site can be considered a social space.

The first area is located immediately next to the primary access. This area uses the lighting poles from the previous location with a modern-futuristic look. A swing with the same design accompanies these poles. The purpose of this area is to provide a fairy-tale setting for taking photographs.

The second area is located on the lakeshore. Here three alveoli are created to allow the placement of high bar tables. Curly willows (*Salix matsudana* `Tortuosa`) that provide decoration with their branches and architectural shape replace the old willows.

The design of the primary access was developed based on the initial sketch. The shape of the light arches was modified to reproduce the forms of lotus flower petals, the predominant shape in the new building design.

Along the entire length of the central alley, on the left side, a relatively large portion of the lawn has been proposed in the shape of an arc. Perimeter to this shape, five specimens of staghorn sumac (*Rhus typhina* `Dissecta`) have been placed on the round side. These species will provide decoration throughout the year through their architectural form, the autumn leaves, and the red flowers that will remain on the branches during winter. The physical characteristics of the land, such as its location, size, surface area, shape, topography, plot, access to utilities, soil features, and local climate, were considered during the systematic planning of the plant arrangement on the site. This was an essential factor in ensuring the

appropriate selection of plants and their suitability to the site's environmental conditions (Pascu et al., 2022). Depending on the light available in the different areas of the garden, the plant compositions will be made according to this criterion.
The garden design will include specific shade-tolerant plant species in areas with more shade. Some examples of shade species that will be used in such areas are Japanese barberry (*Berberis thunbergii* `Atropurpurea`), summer lilac (*Buddleja davidii* `Purple Prince`), Sawara cypress (*Chamaecyparis pisifera* `Baby Blue`), and smoke tree (*Cotinus coggygria* `Royal Purple`). Other suitable plants for shaded areas include hostas, ferns, astilbes, and heucheras. These plants were chosen based on their ability to thrive in areas with limited direct sunlight and can enhance the aesthetic appeal of the shaded regions in a garden.
The chosen plants mainly provide decoration through leaves and habit, especially shrubs and subshrubs. To mask certain areas or provide privacy from the street, taller shrub species have been proposed, species that will also offer decoration through flowers to liven up the whole design, such as summer lilac (*Buddleja davidii* `Purple Prince` and `White Ball`), smoke tree (*Cotinus coggygria* `Royal Purple`), lilac (*Syringa vulgaris* `Kathrine Havemeyer`), salt cedar (*Tamarix ramosissima* `Rubra`) and European cranberry bush (*Viburnum opulus* `Roseum`).
In addition to the listed shrubs, other plants that are suitable for sunny areas include perennial flowers such as orange coneflower (*Rudbeckia fulgida* `Goldsturm`), purple coneflower (*Echinacea purpurea* `Magnus`) and woodland sage (*Salvia nemorosa* `Caradonna`).

Control, revision, and approval

The control, revision, and approval process, including landscape design, is essential to any design project. As Nijhuis and de Vries (2019) noted, this process is critical for ensuring that the design meets the needs and expectations of the beneficiaries involved.

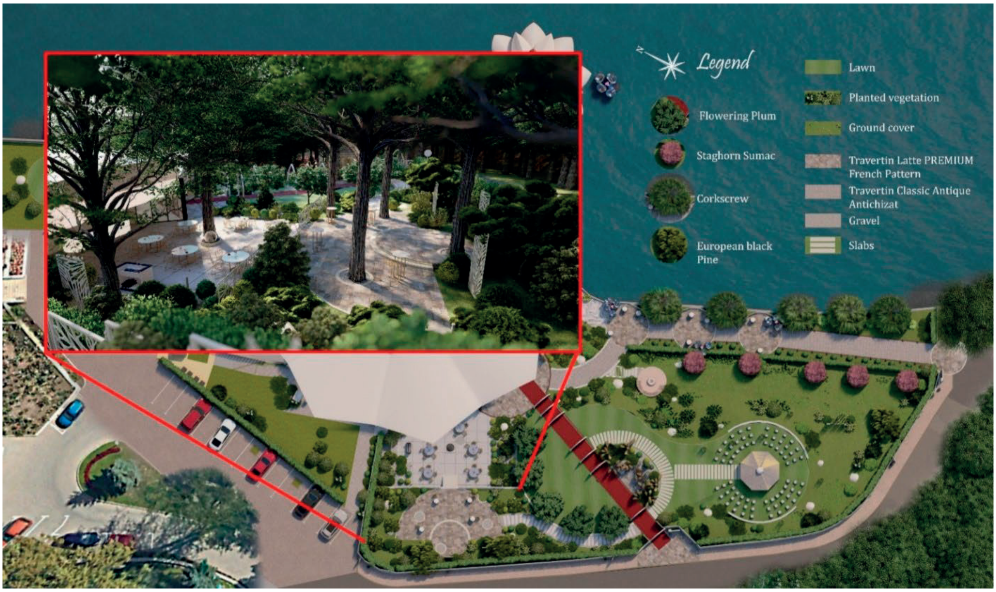

Figure 7. Final design project - plan view

In the case of the garden design project, the feedback received highlights the importance of revisiting the preliminary design to ensure that it aligns with the beneficiaries' expectations. The desired changes, such as adding more vegetation, reducing terrace shapes, and eliminating themed alveoli, indicate the beneficiaries' preferences for a more natural and simplistic design.
Moreover, the desire for an additional access point to the upper terrace of the lounge area emphasizes the importance of considering the

user's needs and accessibility in the design process. Nijhuis and de Vries (2019) emphasize the importance of user-centered design, where the users' needs and preferences are considered throughout the design process.

In summary, the feedback received during the control, revision, and approval stage highlights the importance of revisiting the preliminary design to ensure it meets the beneficiaries' needs and preferences. Incorporating user-centered design principles and considering accessibility can improve the overall design's functionality and user experience.

Final design project

The design of the lounge area underwent significant modifications following feedback from beneficiaries. The necessary improvements implemented were removing thematic elements and introducing a semicircular terrace for dining purposes (Figure 7). The preservation of existing healthy trees was prioritized in the design, with recommended pruning techniques aimed at rejuvenating tree crowns and eliminating dry branches. Replacing sick trees or those at risk of collapse with decorative trees was undertaken where necessary.

Despite the rigorous evaluation phases that preceded the design modifications, some elements only became apparent during implementation. These included soil impurities, significant level differences, large tree roots, and vegetation injury. As such, the design underwent further minor changes during the performance, with on-site assistance from the landscape engineer responsible for the project deemed necessary.

Given the dynamic nature of the design process and the need for careful oversight during implementation, the landscape engineer in charge of the project will be available to provide on-site service at critical implementation moments. An automatic irrigation system will also be installed throughout the landscape to ensure optimal plant health and growth.

CONCLUSIONS

Designing a private events venue garden involves various factors, including the purpose of the events, location, existing vegetation, and style. The entire design process should be carefully planned, with each step being essential and treated with great care and seriousness.

To achieve a beautiful and functional garden, the design should consider a sinuous style that follows the building's new design and blends harmoniously into the area’s natural landscape. The existing vegetation should also be considered, and minimal intervention should be made. Where trees need to be removed, they should be replaced with trees of the same species or decorative trees.

The plant compositions in the garden should mainly consist of decorative species with leaves, while taller shrub species can provide decoration through flowers. The selection of plant species should be based on various criteria, such as suitability to the local climate, water requirements, and maintenance needs.

Research on similar projects has identified essential factors such as the use of native plants, the incorporation of water features, and the creation of multi-functional spaces. These factors should be incorporated into the design to achieve a beautiful and functional garden.

Overall, the design process of a private events venue garden requires a balance between functionality and aesthetics while considering the users' needs and preferences. Designers should also incorporate principles of sustainable design to create a garden that enhances the overall experience for event attendees.

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INFLUENCE OF ROOTING MEDIA AND HORMONES ON *MANDEVILLA* VEGETATIVE PROPAGATION

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Abstract

Ornamental flowering plants are used worldwide for landscape design and as cut flowers. Most are imported from abroad and climatic conditions are different in their countries of origin. Although they produce seeds, these are often sterile and cannot germinate. Therefore, such species are multiplied vegetatively. In the present study it was investigated the propagation of two varieties of Mandevilla sanderi under the effect of three different rooting hormones (Incit 8, Radi-Stim® nr.1, Radi-Stim® nr.2), and two different rooting media (peat-perlite and peat). Untreated cuttings were considered as control. The results indicate that significant changes were obtained under the effect of the selected rooting stimulants, and that the rooting media did not influence the root initiation of the cuttings. Furthermore, no significant changes were observed on cuttings growth after transplantation. In conclusion, the current research shows that I8 and R2 increase the rooting of Mandevilla cuttings, and that the rooting media have no effect on the plants.

Key words: *climbing plant, cuttings, Mandevilla, stimulants, propagation.*

INTRODUCTION

Floriculture is part of the horticultural industry and encompasses the production of flowering and ornamental plants for landscaping and floristry (Paiva, 2018; Wani et al., 2018). Floriculture in recent years has become the fastest growing industry in the commercial horticulture trades, due to people's rising living standards and demands (Manikas et al., 2020). With the increasing demand, flower nurseries need to find new and efficient/fast methods to propagate different species.

The genus *Mandevilla* Lindl. belongs to the family *Apocynaceae*, and is native to the American continent, with 191 species (The Plant List, 2021). It includes flowering climbing/liana species, and some shrubby herbs or epiphytes (de Sales et al., 2006). Plants of this genus are beloved as ornamentals in pots or containers, and are excellent for indoors or on balconies decoration (Kozak et al., 2019; Kozak et al., 2021). Because of the beauty of their flowers, *Mandevilla* species, known as rock trumpets, are among the most widely cultivated ornamental plants worldwide (Cordeiro et al., 2012).

Mandevilla sanderi (Hemsl.) Woodson (syn. *Dipladenia sanderi* Hemsl.), is known under its commercial name of "Brazilian jasmine" (Oder et al., 2016). At least 50 *Mandevilla* species are native to Brazil (Cordeiro et al., 2011; Favara et al., 2020). *M. sanderi* is a vine with funnel-shaped pink, white or red flowers, blooming between late spring and fall.

The species can be propagated by seeds, stem cuttings and also by micropropagation. Seed germination has the disadvantage that it requires favourable environmental conditions and seed-propagated plants may not retain the characteristics and genetics of the mother plants, and obtaining mature plants would take much longer. On the other hand, *M. sanderi* can be multiplied by micropropagation, with a high production rate of new plants (Cordeiro et al., 2014a; Cordeiro et al., 2014b). However, this type of propagation requires laboratory equipment and is too expensive for smaller nurseries to purchase. Vegetative propagation ensures that all characteristics of the parent plant are retained. The success of vegetative propagation is mainly affected by environmental factors, the quality of the growing medium and rooting hormones (Faust et al., 2014).

Plant hormones are naturally present in plants, although in low concentrations, and are organic substances that influence the physiological process, thus influencing plant functions and development, such as rooting, fruit ripening and plant growth (Mirihagalla & Fernando, 2020; Anfang & Shani, 2021), they may also have an effect on stomatal movement (Davies, 2010).

In floriculture, hormones are often used to stimulate root initiation of cuttings (Faust et al., 2014), on the other hand this process provides the basis for clonal multiplication of plants (Druege et al., 2016). Adventitious root formation is a critical phase for the survival and development of unrooted cuttings (Koroch et al., 2002), and involves biological, morphological and even physiological changes (Geiss et al., 2009; Zhang et al., 2017).

The present study investigated the influence of three rooting hormones commonly used in Romanian floriculture (Incit 8, Radi-Stim® nr. 1 and Radi-Stim® nr. 2) and of different rooting media (peat-perlite and peat) on two *M. sanderi* varieties. The influence of the hormones on rooting percentage, number of roots, root length, root volume was investigated, and their effect on the growth of plants, as only little information is available on this species vegetative propagation, especially with rooting hormones. We aimed to determine which rooting hormone and growth media are most suitable for the vegetative propagation of this species.

MATERIALS AND METHODS

Plant material and study site

The experiment was carried out between 22 March and 21 July 2021 in a commercial greenhouse with controlled temperature and humidity, located at Mureș County, Sărățeni (46°33’37.6” N 25°00’43.1” E). The mother plants were obtained from a local garden centre. Two varieties of *Mandevilla sanderi* were selected: ‘Classic Red’ (Red) - red flowers and ‘Classic White '16’ (White) - white flowers, both valuable as ornamental pot vines. The two varieties have medium-early flowering and are suitable for hanging pot cultivation. Apical cuttings were harvested from the mother plants and immediately immersed in the rooting hormones and planted in the specific growth media for each treatment. Humidity and temperature were measured using a Testo 175H1 (Testo Romania, Cluj-Napoca, Romania). Recorded humidity during the experiment was 70-95%, and the average temperature 16.87 °C (Figure 1).

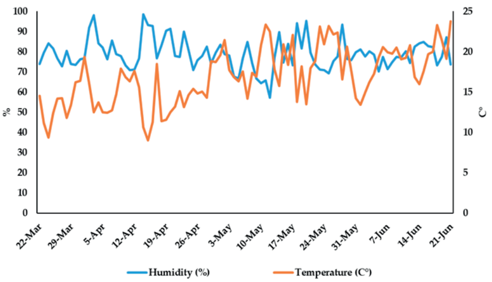

Figure 1. Average humidity and temperature during the greenhouse experiment

Growth media and rooting hormones

As substrates a mixture of peat-perlite (PP) (50-50%) and simple peat (P) were compared. The peat used in the experiment was Ageresti OÜ (Pärnu, Estonia). The pH of the substrate was 5-6.5, the fraction between 0 and 20 mm, and N:P:K content 14:10:18. Yet, we have supplemented the N:P:K content with 50-260 mg/L of N, 50-260 mg/L P_2O_5, and 50-340 mg/L of K_2O. The perlite granule size was 6 mm.

The selected rooting hormones for the treatments were Incit 8-I8 (0.8% 1–Naphthaleneacetic acid) (AMVAC Chemical UK Ltd., Surrey, UK), Radi-Stim® nr. 1 - R1 (CCDB Bios, Cluj, Romania), and Radi-Stim® nr. 2 - R2 (CCDB Bios, Cluj, Romania). *Mandevilla* cuttings without treatment were considered as control. The rooting hormones were selected because of their availability on the market for ornamental plants producers and for their successful use in previous studies for vegetative propagation (Panea et al., 1997; Vlad & Vlad, 2008; Jeberean & Bala, 2017; Vlad et al., 2019; Boboc et al., 2020).

Experimental design

On 17 May 2021 the cuttings from the first experiment were transplanted into 0.3 L pots in the previously mentioned growth media (PP and P), plants from the second experiment were transplanted on 7 July similarly to the first. After transplantation the plants were watered alternately with tap water and Universol Green 23-6-10 complex fertilizer (Holland Farming Agro SRL, Bucharest, Romania).

Data evaluation

Rooting percentage (% of the cuttings that developed at least one root), root volume (cm^3), number of roots, root length (cm) measurements were collected on the day of transplantation in larger pots of the cuttings. The volume of the roots was determined by filling a test tube with water in which the cuttings were immersed, and the volume of water displaced was measured. For both propagation periods growth (cm) measurements were made on day 0 on day 15 after transplantation.

Statistical analysis

Data were analyzed using Past 4 statistical software (Oslo, Norway). Data were tested for normality of errors and homogeneity of variance. All data were normally distributed. The significance of the differences between the treatments was tested by applying ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$.

Bars in the figures represent the means ± SE (n = 30). Different lowercase letters above the bars indicate significant differences between treatments, and uppercase letters indicate the significant differences between the two–growth media (Peat-Perlite and Peat), according to Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSIONS

Rooting percentage

The rooting percentage of the *Mandevilla* varieties was affected in different ways by the applied rooting hormones (Figure 2). Small increases were observed in the red flower variety (peat-perlite growth medium) in the first propagation, under the influence of I8 and R2, on the other hand an inhibition of rooting percentage was recorded in the cuttings under the R1 treatment. However, in the peat substrate, high increases were recorded under the hormone treatments compared to the control (Figure 2a). According to the results obtained in PP, a small inhibition was observed in plants subjected to rooting hormones, however, in the P substrate significant increases were detected in the I8 and R2 treatments (Figure 2b). Here too, an increase in cuttings was observed under treatment I8 in PP growth medium, but no significant differences were recorded in P (Figure 2c). In white *Mandevilla* cuttings (second propagation) high increases in all three rooting hormones were observed in PP, on the other hand there was no significance in P (Figure 2d). An increase in rooting percentage was observed in the second propagation period among the white *Mandevilla* controls (Figure 2d), the root fitness of the cuttings increased significantly in the peat substrate. However, in the other results no differences were found when comparing growth media.

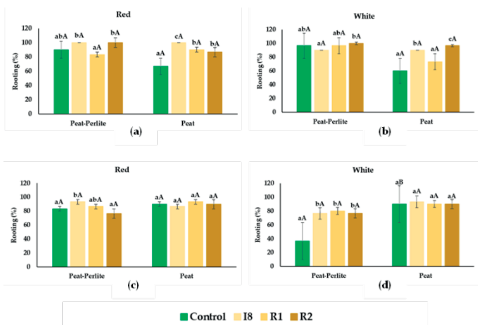

Figure 2. Influence of rooting stimulants (I8 - Incit 8; R1 - Radi-Stim® nr. 1; R2 - Radi-Stim® nr. 2) on rooting percentage of *Mandevilla*: (**a**) 'Classic Red' first propagation; (**b**) 'Classic White 16' first propagation; (**c**) 'Classic Red' second propagation; (**d**) 'Classic White 16' second propagation

Number of roots

As expected, the root number was significantly affected by the rooting hormones (Figure 3). A high increase in cuttings was detected in PP under R2 compared to the control. On the other hand, the number of roots of *Mandevilla* in peat was strongly influenced by all three hormone products (Figure 3a). No significant differences were determined between the control and the I8 treatment, but R1 and R2 greatly increased the number of roots in the PP growing medium. On P substrate, in the first propagation period, the number of roots of white *Mandevilla* cuttings was significantly increased by I8 and R2 hormones compared to the control (Figure 3b). In the second propagation period, the number of roots of red *Mandevilla* cuttings increased with I8 and R2 in peat-peat moss, however in peat significant differences were determined only in R2 (Figure 3c). Root number was greatly improved in the I8 treatment and in PP growth medium; no significance was observed in the other two treatments. A small inhibition of root number was recorded in R1/P compared to the control, also I8 and R2 increased root number, but by a small percentage (Figure 3d). Significant differences between growth media were observed in the second propagation period in the white cuttings (Figure 3d); in the peat substrate the number of roots was significantly higher than in peat-perlite only in the control and R2 treatments.

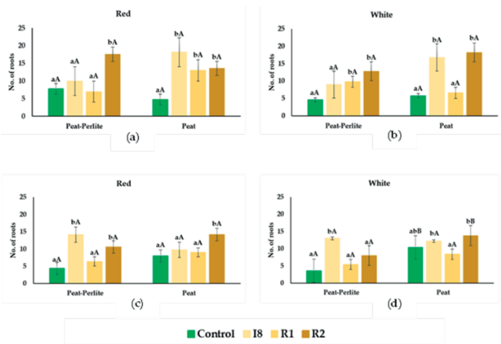

Figure 3. Influence of rooting stimulants (I8 - Incit 8; R1 - Radi-Stim® nr. 1; R2 - Radi-Stim® nr. 2) on number of roots of *Mandevilla*: (**a**) ‘Classic Red’ first propagation; (**b**) ‘Classic White 16’ first propagation; (**c**) ‘Classic Red’ second propagation, (**d**) ‘Classic White 16’ second propagation

Root length

Regarding root length, the hormones influenced the root growth in different ways, although in most cases their effect was positive (Figure 4). In the first propagation trial, rooting hormones increased the root length of the cuttings in all treatments/culture media compared to the control (Figure 4a). Root length of white cuttings was also positively affected under the treatments in both substrates; however, in the peat substrate no significant differences were found between control and R1 (Figure 4b). In the second propagation period, root length of red *Mandevilla* cuttings only increased in I8 and R2 on PP growing media, and in peat under R1 and R2 treatments (Figure 4c). As for the white cuttings in the second propagation period, their root length increased significantly with all three hormones compared to the untreated ones in PP. However, only small increases were observed in the peat substrate between treatments, and R1 even decreased root length (Figure 4d). When comparing growth media, increases in root length were detected in red cuttings in the second propagation period (Figure 4c), but only in the R1 treatments; root volume increased greatly in peat growth media compared to peat-perlite.

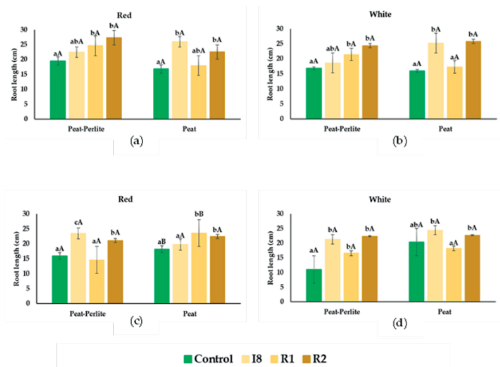

Figure 4. Influence of rooting stimulants (I8 - Incit 8; R1 - Radi-Stim® nr. 1; R2 - Radi-Stim® nr. 2) on root length of *Mandevilla*: (**a**) ‘Classic Red’ first propagation; (**b**) ‘Classic White 16’ first propagation; (**c**) ‘Classic Red’ second propagation; (**d**) ‘Classic White 16’ second propagation. Bars represent the means ± SE ($n = 30$)

Root volume

As expected, root volume significantly increased in almost all treatments compared to control (Figure 5). In the first propagation experiment rooting hormones highly increased the root volume of the *Mandevilla* cuttings on both growth media (Figure 5a). Similar to the previous results significant differences were determined when comparing the treated and untreated cuttings (Figure 5b). Under our experimental conditions significant differences between the growth media were observed in the second propagation time: the red variety´s root volume considerably increased on peat-perlite substrate in cuttings treated with I8 (Figure 5c) and that of white *Mandevilla* increased significantly on peat in the untreated plants (Figure 5d). No significant differences were found between the cuttings treated with R1 in PP, but the application of I8 and R2 highly increased the root volume. Although cuttings planted in peat showed an increased volume when subjected to the selected treatments (Figure 5c). In the white variety planted on PP growth medium, significant differences were determined when comparing I8 and R2 treated cuttings with the control, however in P only in the R2 treatment were found increments compared to the untreated cuttings (Figure 5d).

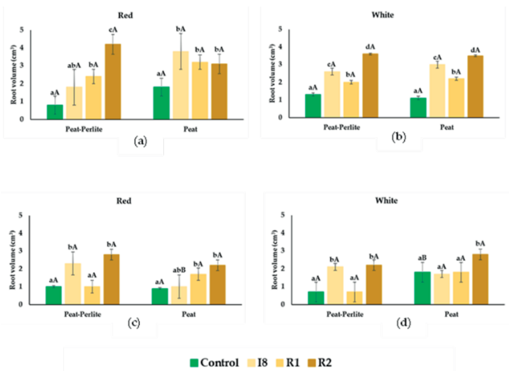

Figure 5. Influence of rooting stimulants (I8 - Incit 8; R1 - Radi-Stim® nr. 1; R2 - Radi-Stim® nr. 2) on root volume of *Mandevilla*: (**a**) ‘Classic Red’ first propagation; (**b**) ‘Classic White 16’ first propagation; (**c**) ‘Classic Red’ second propagation; (**d**) ‘Classic White 16’ second propagation

Growth

It was observed that rooting hormones can influence growth only to a minor extent (Figure 6). In the red *Mandevilla* cuttings from the first propagation experiment, only in the growth medium PP R1 did the growth of the cuttings increase significantly (Figure 6a). In the white cuttings, no significant differences were found in any of the treatments or growth media after 15 days of observation (Figure 6b). In the case of red cuttings in the second propagation period, significant increases were only recorded in peat-perlite in the R2 treatment; no significant differences were determined in the other variants (Figure 6c). Similar to the previous results, no significant changes were recorded in PP, and in P only growth inhibition was observed in treatment I8 compared to untreated cuttings (Figure 6d). Comparing the results of the growth media, it can be stated that the growth of cuttings in peat-perlite exceeded that of cuttings planted in peat only (Figure 6d).

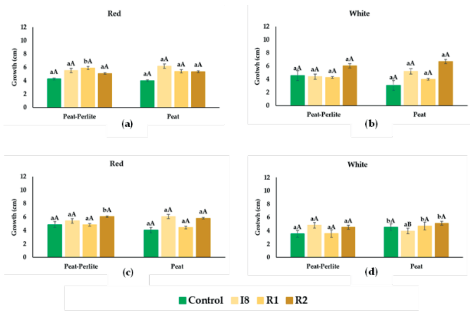

Figure 6. Influence of rooting stimulants (I8 - Incit 8; R1 - Radi-Stim® nr. 1; R2 - Radi-Stim® nr. 2) on growth of *Mandevilla*: (**a**) ‘Classic Red’ first propagation; (**b**) ‘Classic White 16’ first propagation; (**c**) ‘Classic Red’ second propagation; (**d**) ‘Classic White 16’ second propagation

The results of the present study indicate that appropriate rooting stimulants can have a positive influence on the rooting of Brazilian jasmine. Rooting hormones can influence the rooting ability of different ornamental plants to obtain maximum percentage of rooted cuttings in a short period of time (Chaudhari et al., 2018; Kumar et al., 2019; Kumar et al., 2020; Kaushik & Shukla, 2020; Sao, 2021). The addition of external hormones to unrooted cuttings can influence and accelerate the rooting process of the plants and may even influence plant growth. Rooting hormones application can improve recovery of the wounded surface, also can influence a rapid appearance of adventitious roots, which can highly increase the survival rate (Kentelky et al., 2021).

Growth and development of plants are affected by different factors, among which rooting media is one of the most vital factors which can have a major role in the adventitious rooting appearance (Kumar et al., 2019). For proper rooting and growth, the substrate must provide water, supply nutrients, permit gas exchange and should also support the plant (Younis et al., 2007; Waseem et al., 2013). Our findings indicate that the growth media in some cases induced changes in the measured parameters, but no distinguishable results were found, so we cannot state that one of the substrates is more efficient than the other for the rooting process. Therefore, we can assume that for rooting of *Mandevilla* both types of growth media are optimal to be used for vegetative propagation.

From our results, it can be concluded that the hormones enhanced the rooting percentage of the two *Mandevilla* varieties. However, it is important to mention that in the second propagation period in the peat growth medium there was no significant increase in the treated cuttings compared to the untreated ones. This could be explained by an effect of the planting substrate, with better peat retaining moisture better and ensuring a higher amount of nutrients for the unrooted cuttings. In a previous study Radi-Stim was reported to increase the rooting percentage of Campsis and Lonicera cuttings compared to another stimulator (Jeberean et al., 2016).

The application of rooting stimulants increased the number of roots; however, I8 and R2 induced greater increases than R1. In previous studies it was reported that Radi-Stim® nr. 2 increased the number of roots of *Laurus nobilis* by 85% compared to the control (Vlad & Vlad, 2008). Also, it was determined that 0.001% of 1-Naphthaleneacetic acid clearly increased the rooting percentage of *Ficus benjamina* L. (Topacoglu et al., 2016).

The data obtained show that rooting stimulants can have a clearly positive effect on the root length of *Mandevilla* cuttings. In our experimental conditions, I8 and R2 greatly increased root length in both varieties and propagation periods. On the other hand, the rooting hormone R1 did not report such a high increase, however in the first propagation period the red and white cuttings on PP substrate and in the second propagation experiment the red ones on P and the white ones on PP induced an increase in root length compared to the control. Previously Radi-Stim was found to positively influence the root length of *Bougainvillea brasiliensis* (Vlad et al., 2009).

The root volume of the cuttings was affected by the rooting hormones used, especially in the first propagation period, where all three stimulants increased the root volume in the two varieties. In the second propagation period, on PP substrate, only I8 and R2 showed significant effects and on peat the two Radi-Stim products determined significant changes. A similar influence was observed in peat-perlite on white variety's cuttings, although in peat only R2 increased the root volume. Previous studies have also reported great increases in root volume under different stimulants (Asănică et al., 2017).

From our results, it can be concluded that the rooting stimulator did not have strong effect on growth after 15 days. R1 increased the growth of the cuttings only in the PP growth medium in the red *Mandevilla* cuttings in the first propagation period. A positive influence was also observed in the second propagation period in the red cuttings in the same substrate, but in this case in the cuttings treated with R2. In the second propagation period of white cuttings in peat, an inhibition was determined in plants subjected to rooting hormone I8. Altogether, can be concluded that stimulants could have positive/beneficial effects on the rooting and survival rate of the cuttings (Nita & Iancu, 2009; Ghosh et al., 2017; Kumar et al., 2020).

CONCLUSIONS

The present study provides data on the comparison of three rooting hormones and two rooting media on the propagation of two varieties of *Mandevilla sanderi*. According to the results, it can be concluded that Incit 8, Radi-Stim® nr. 1 and Radi-Stim® nr. 2 have a positive effect on rooting of cuttings, but mostly when cuttings were treated with I8 and R2. These two rooting stimulants had a greater effect on the rooting percentage of the cuttings and also on the other measurements. However, none of the applied treatments showed any effect on the growth of the cuttings. With regard to the growth media, it was found that peat-perlite and peat did not influence the initiation of the roots of the cuttings or their growth. Regarding the differences between the two varieties could be concluded that in most of the case both varieties recorded almost similar data, although the 'Classic Red' variety growth was above the 'Classic White 16'.

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ECOLOGICAL EDUCATIONAL TRAIL, AS LINKAGE BETWEEN NATURAL AREAS AND HISTORICAL HERITAGE, PRESENTED BY THE CASE STUDY OF PĂNET VILLAGE

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Abstract

With the expansion and overcrowding of cities, there is an ever-increasing demand and need for "close to nature" areas, which provide recreation and relaxation. The aim of the study is to present a method for the creation of an ecological educational trail, which can link the natural places with the local historical heritage of settlements in Transylvania. Educational paths highlight the values of the past and transfer it in such an actuality and modern way that can be approached from several sides. This symbolic linkage created by the educational trail is between the present represented by the natural areas, and the church and the central areas next to it representing the historical heritage, as the past. The planned educational trail is located in the Pănet settlement, in the Transylvanian plain, 11 km from Târgu Mureș. The goal is to re-think and recall the very close connection between humans and nature, as in the old days. For this purpose, the educational trail proves to be a promising solution given this rich vegetation and natural features in the area.

Key words*: ecological educational trails, livable places, sustainable city.*

INTRODUCTION

The experience of being surrounded by nature is truly remarkable. Some poetic lines aptly capture the essence of this sensation by alluding to the eternal beauty and mystery that nature embodies. These two qualities are what natural environment render so significant.

Unfortunately, many individuals perceive this importance to be a malleable concept, which leads to widespread ignorance. Consequently, both the beauty and mystery of nature are at risk of being compromised. It is possible to instill a sense of significance for nature through deliberate education and nurturing. Two key qualities that can serve as effective tools in this endeavor are yet to be identified, and their identification is the primary motivation for this research.

As cities expand and become more crowded, there is an increasing demand for areas that are "close to nature" provide recreation and relaxation for both local residents and visitors. The avalanche of environmental issues in recent years has intensified the use of environmental education as a means to inform and instruct individuals regarding the dangers of pollution and the deterioration of ecosystems (Ribeiro et al., 2012; Karatas & Karatas, 2016). Thematic educational trails can effectively fulfill the need to raise awareness about environmental issues. In addition to providing opportunities for active recreation and relaxation, these trails can also serve as means of drawing attention to the significance of environmental factors, educating visitors about ecological principles, and promoting nature-based tourism (Peter & Cheruto, 2013). Nature-based tourism is commonly acknowledged as a rapidly growing sector within the world's largest industry, and serves as a crucial bedrock for conservation. Although visit rates -assessed by two distinct methodologies- are indeed decreasing in certain affluent countries, data from approximately three-quarters of nations where such information is available indicate that visit to protected natural areas is on the rise (Balmford et al., 2009).

The aim of the study is to present a method for creating an ecological educational trail that links natural areas with the local historical heritage of settlements in Transylvania. This symbolic linkage created by the educational trail connects the present, represented by the natural areas, with the past, represented by the church and central areas nearby.

The planning of the educational ecological trail is based on the theory of Shamala M.M., (2020). According to her theory, the ecological trail emphasizes that humans are guests, not masters, of nature and should respect the rules of the natural environment. This approach encourages the protection, appreciation, and observation of nature, recognizing that humans are an integral part of it (Shamala, 2020).

There are numerous studies worldwide emphasizing the significance of preserving the flora and fauna along the route of ecological educational trails (Blanco et al., 2021; Silva et al., 2019; Dunkley, 2016). One of the primary objectives of the planning process was to emphasize this issue. The design of the central part clearly depicts the connection between the natural fields and the “soul” of the village, with religious belief, community, and memory being the main pillars of society.

The ecological educational trail presents a promising solution, leveraging the rich natural features and vegetation in the region. Moreover, this project provides an opportunity to promote nature-based tourism, which can significantly contribute to the settlement’s economic development. The trail creates and represents a valuable resource that effectively highlights the region's natural beauty. The areas surrounding the trail host a diverse range of spontaneous flora, herbaceous plant associations, and mature trees, all of which have been largely untouched. To minimize any disturbance to the natural areas, it is crucial to avoid introducing additional plants along the trail. Rather, the focus should be on preserving the existing natural environment by taking as little as possible from these pristine areas. Educational trails offer a contemporary approach to the transmission of values from the past and presents various perspectives.

METHODOLOGY

The aim of this study is to determine optimal strategies for establishing a symbiotic relationship between humans and nature, with the goal of providing ecological, economic, and social benefits.

The research methodology comprises two primary components. Firstly, the proposed subject is contextualized through a literature review of the related site, focusing on two perspectives: preservation of nature and constructed heritage. Secondly, a comprehensive analysis of the territory is conducted, involving multiple map documentation and a case study of the planned educational trail that links the main objectives.

The multifaceted planning program encompasses two main components. The first component is the central square, which will be created by connecting the churchyard and the memorial square. The second component is the educational trail, which covers the eastern portion of the administrative unite of the settlement.

LITERATURE REVIEW

There is currently no consensus on a universally accepted definition of educational trails, which may be attributed to the variability of the nature trail concept across different countries and the potential difficulty in distinguishing it from cultural or nature walkways. Additionally, it may be challenging to determine when a thematic path can be classified as a nature trail. According to Hungarian literature, an educational trail is typically characterized by a designated thematic route with marked stations featuring information boards (Fodór, 2018).

In addition, the educational trail must also address social needs and serve as a means to satisfy ecotourism functions or provide an alternative but meaningful way to spend leisure time, if applicable (Kollarics, 2015).

The educational trail can be viewed as a path that connects various stations, featuring ecological attractions along the way (Bajor-Lampert & Bajor, 2018). "Eco-attraction" is a term that has emerged from the field of ecotourism studies and refers to natural features or phenomena that are appealing to visitors (Orams, 1995).

The genesis and evolution of educational trails have a fascinating history marked by cause and effect, which aptly reflects the significance of positive reinforcement of the nexus between humans and the environment in shaping consciousness, not only in contemporary times but also back from the beginning of the 20th century (Kollarics, 2015). Nature trails have a significant role in the sphere of social life, particularly concerning nature and its conservation. Unfortunately, these concepts are not well understood by the public nowadays. Hence, it is essential to address this issue since numerous valuable species still exist, not only in rural areas but also in urban regions (Bajor-Lampert, 2014).

Despite the significant developments in the planning and design of educational trails in the 21st century, poorly executed plans are still prevalent, including in Central-Eastern Europe (Fodór, 2018). Ecotourism, a widely utilized economic diversification tool in developing countries, is commonly perceived as a means to protect ecosystems, preserve local cultures, and spur economic development (Che, 2006).

On the topic of education, it is important to emphasize the significance of meticulous planning when designing an educational trail, and to consider the objectives that it aims to achieve. One of the primary considerations is the age group of the intended audience, which should inform the selection of relevant topics that align with the educational and pedagogical framework. According to surveys, the ideal length for an educational trail is between 1 to 1.5 hours. In areas with undisturbed nature, the use of materials and the extent of intervention should be carefully considered when designing the trail (Kiss, 1999; Fodór, 2018). The scope and form of information dissemination varies depending on the target audience and the subject matter. There are various approaches to convey the abundance of information surrounding educational trails, ranging from basic signage to advanced technological solutions (Kollarics, 2015; Fodór, 2018).

URBAN DEVELOPMENT

The research regarding to the proposed ecological educational trail is located in Pănet, a village situated in the Transylvanian plain approximately 11 km from the city of Târgu Mureș and 6 km from the Mureș River. (P.U.G., 1998). The hills in the area have a height ranging from 325 to 425 meters, with the lowest point being 315 meters above sea level. It is worth noting that the broad ridges and gentle slopes seldom exceed 14 -16 degrees, except for a few hills around Târgu Mureș that can be steeper, with an angle of up to 40 degrees (Konrád, 1996). This area, which is also referred to as "the gate of the Transylvanian plain" (Nagy, 2008), is a valley featuring a wooded area that exhibits a rich variety of flora and fauna. The historical evolution of the core part of the village, based on military survey and maps, is illustrated in Figure 1.

The study site is situated in a region characterized by forests and fields, and has traditionally served as a hub for regional agriculture, forestry, and hunting activities (Konrád, 1996). To this day, the area remains a valuable destination in nature, with the vibrant spring pheasant's eye (*Adonis vernalis*) blooming in the early season and the snake's head, or checkered lily (*Fritillaria meleagris*) still prevalent in certain areas, offering ample opportunities for exploration.

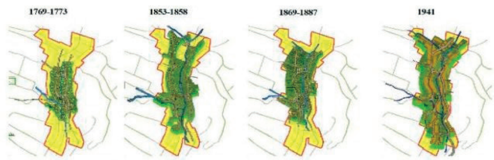

Figure 1. Urban development of the settlement Pănet (based on: Arcanum Digitheca military surveys)

RESEARCH AREA DESCRIPTION

1. Natural heritage - presentation of the proposed ecological trail's area

The educational trail is planned to be situated in Pănet, 11 kilometers away from Târgu Mureș city, a highly urban area. The distance and accessibility of the trail area from the city are optimal for a one-day excursion in nature. The area can be reached by car, bus, or even with bicycle, providing a delightful opportunity for a day out in nature, away from the crowded urban areas.

Its objective is to revive the intimate bond between humanity and nature, reminiscent of bygone times. The area's luxuriant flora and natural characteristics make the educational trail an auspicious measure for achieving this aim.

The proposed educational trail area covers a section of approximately 375 hectares in the eastern outskirts of Pănet, representing roughly one quarter of the entire region. This area is primarily utilized for agricultural, forestry, and grazing activities, leading to the formation of an extensive network of agricultural roads. Despite significant human activity, the area retains a diverse range of flora, including numerous herbaceous plant species such as *Adonis vernalis*, commonly referred to as the spring pheasant's eye. However, certain species, such as the checkered lily (*Fritillaria meleagris)*, have become increasingly rare, as research indicates. The eastern section of the area also features several old trees, including nearly century-old silver birch (*Betula pendula*) and Norway spruce (*Picea abies*) located in the churchyard. Additionally, the area boasts a white poplar (*Populus alba*) that qualifies as a "Methuselah tree" with a trunk circumference of nearly 400 cm and a height of over 20 m (Posfai, Gy., 2005). The vegetation analysis of the area reveals a diverse range of flora, which indicates that the location is suitable for an ecological trail. This finding underscores the need for further research and planning of the trail.

The visual assets in the area are not only visually appealing but also hold significant cultural and historical importance. For instance, Capele hill stands as a testament to a World War II event, while on a clear day, the summit of Becheci can be observed in the far north-east. These features, along with the hilly terrain, provide excellent panoramic views, enhancing the trail's educational value. Overall, the rich natural characteristics, high level of biodiversity, and chromatic diversity of the area make it a prime candidate for the development of an educational trail.

2. Historical heritage - presentation of the central area

Religious belief, faith have played a significant role in shaping the historical and cultural landscape of the village. The original inhabitants of the area, the Szeklers, a regional Hungarian speaking community, were initially mostly Roman Catholics, later become Reformed.

The precise construction date of the church remains unknown, although several indications suggest that it was built in the 15th century. The southern door's design bears a striking resemblance to that of the Gornești castle nearby, built in the 15th century, leading to the inference that the same skilled stonemason crafted it. Additionally, the church's Gothic architectural style supports the hypothesis that it was constructed during this period (Konrád, 1996).

The Reformed church, built in the 1740s, served as a central point in the settlement, accompanied by a communal well located nearby. However, no other spatial design characteristics that reflect the period's style were observed in the vicinity. Old photographs indicate the presence of trees in the yard, while historical records document the plantation of an orchard, as recorded in the clerical reports. The lack of protection for the historical and heritage value of the memorial buildings in Pănet is a significant issue, given the presence of four monuments (Figure 2), including the Szekler gate constructed in 1787, located in the central area near the Reformed church (Cultural Ministry of Romania, 2015).

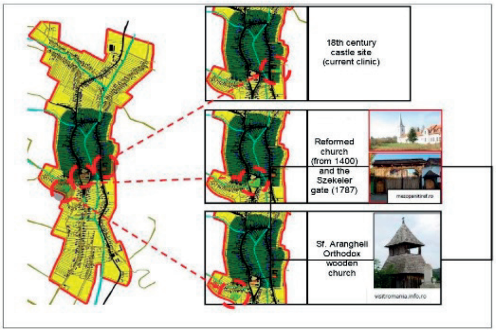

Figure 2. Monuments (according to: General urban plan of Pănet, 1998)

The church courtyard has been utilized only sporadically for religious services, lacking additional functions or frequent usage. To promote spiritual growth and maximize the utilization of the space, it has been deemed appropriate to assign a new function to the area. This decision was motivated by the ongoing expansion of the building, which represents an unprecedented event in the church's history.

The memorial square, which was established in the 1990s adjacent to the Reformed church, functions as a commemorative place. Connecting this area with the church's courtyard would create a central hub, which

would symbolize the essence of religion and the village's past, while also providing a communal space for the locals.
Moreover, linking this space with the ecological learning trail would establish a connection between the present ecological approach and the church's past, thereby promoting a comprehensive understanding of the site's history and its significance in the community.
A relatively extensive green area with limited street furniture and a few trees lies in front of the Local Council building, as indicated by the P.U.G. of 1998. Although the lack of a well-defined central area in the Pănet settlement, both presently and historically, is a significant research concern, the vicinity of the church retains crucial significance within the village boundaries. It is recommended that the central area be suitably designed for leisure activities, with an emphasis on the addition of plantations, particularly woody plants and street trees along the main roads. These recommendations are in line with the guidelines of the P.U.G. (1998).
The proposed design of the central community space must prioritize a close association with the current functions of the area, specifically highlighting the religious and commemorative aspects that are connected to the church and the monument. Additionally, it should support community-building activities while preserving the educational nature of the trail. Achieving this could involve incorporating significant elements on the ecological trails route, such as the common birch (*Betula pendula*) tree located in the churchyard and the remains of the tombstone from 1866.

ANALYSIS

1. Outer area of Pănet

Pănet holds the largest administrative area among the neighboring villages, serving as the central administrative hub for the Pănet locality (refer to Figure 3).

Figure 3. Administrative areas of Pănet settlement (according to: General urban plan of Pănet, 1998)

The plan consists of two primary components, namely the expansion of the educational trail and the rejuvenation of the central community area. The former involves a section of Pănet's catchment area, which is approximately a quarter of the total, but for a more comprehensive and lucid view, it was deemed necessary to examine the entire administrative region spanning over 1,500 hectares. Figure 4 illustrates the land use and economic distribution of the analyzed areas. It is notable that the majority of the western part is primarily allocated to agriculture, extending directly from the village boundary on that side.

Figure 4. Land use distribution (according to: General urban plan of Pănet, 1998)

2. Inner area of Pănet

An in-depth analysis of the plot structure and the land use is needed in order to emphasize the coherence, harmony of the Pănet rural landscape. The lack of urban influence has allowed the traditional peasant village to persist, and its essence is reflected in the appearance of the constructed elements.

3. Analysis of the ecological trail's route

The evaluation of the area where the educational trail is planed is predicated on several crucial factors, including an investigation of the visual linkages, an assessment of the road network, and the identification of sites conducive to the proliferation of plant and animal species, as well as mature trees suitable for designated station points. The preliminary analysis is underpinned by these fundamental parameters, and supplementary inquiries may be incorporated to precisely demarcate the ultimate outcome.
The survey of the road network (Figure 5) in the specified area indicates that all sections of the area are reachable via unpaved roads. ORANGE arrows indicate access from the village, whereas RED arrows denote access from roads outside the village, mainly field roads.

Figure 5. Road network and accessibility (according to: General urban plan of Pănet, 1998)

After studying the distribution ratio of forested and cleared parts in the hilly region, can be evaluated the visual connections of the area. The analysis of visual connections helped in the identification of key points that require establishing connections along the educational trail, while also highlighted areas that may not be relevant from this standpoint.

The analysis of vegetation is a crucial aspect of the educational trail, and thus, particular attention was given to its evaluation. The natural conditions of the area have allowed, facilitated not only the survival, but further development of a spontaneous flora, which renders the place extraordinary, aesthetically pleasing, and intriguing. The distribution of forest and field wood vegetation is presented in Figure 6, revealing that the dominant tree species in the vast forested area is pedunculated oak (*Quercus robur*), often accompanied by hornbeam (*Carpinus betulus*). As the area is located on a hillside, thickets, mainly consisting of single-seed hawthorn (*Crataegus monogyma*) and cornelian cherry (*Cornus mas*), bind the soil on the slopes. Vines are less prominent, whereas woody pastures are found on the hillsides and edges of clearings, where the wild pear (*Pyrus pyraster*) frequently appears. Although white poplar (*Populus alba*), common walnut (*Juglans regia*), and European black pine (*Pinus nigra*) are present in the area, they are scarce and dispersed.

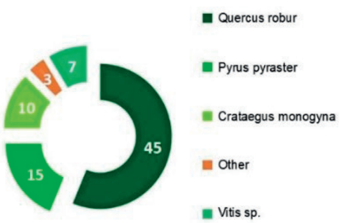

Figure 6. Existing tree species proportion on the ecological trail's area

Regarding herbaceous plants, the environment becomes picturesque, as they usually cover a given area and enhance the human-nature relationship of the settlers. The spring pheasant's eye (*Adonis vernalis*) is particularly noteworthy as it blooms abundantly in the spring, forming a stand on an entire hillside in the northern part of the area, along with the red winter rose (*Helleborus purpurascens*). The forest sites of dogtooth violet (*Erythronium dens-canis*) appear almost everywhere, while in the flatter areas, cowslip (*Primula veris*) form an abundant population.

These plants can be an intriguing focus for the primary segment of a station, not only because of their aesthetic appeal but also due to their potential medicinal properties. Additionally, the broad-leaved Solomon's seal (*Polygonatum latifolium*), greater stitchwort (*Stellaria holostea*), early dog-violet (*Viola reichenbachiana*), *Veronica* sp., the purple gromwell (*Lithospermum purpureo-ceruleum)*, common wormwood (*Artemisia absinthium*), and snowdrop (*Galanthus nivalis*) form dense and large stands, while the checkered lily (*Fritillaria meleagris*), found in only one place, and the greater pasque flower (*Pulsatilla grandis*), found on a few square meters of a hillside in the southern part of the area, are the rarest plants in the surroundings of the settlement (Soó & Kárpáti, 1968). These plants need proper protection (Figure 7).

Figure 7. Herbaceous plants

Various species of butterflies can be observed in the area, including the *Papilio machaon*, *Inachis io*, and *Iphiklides podalaria*. The bird fauna includes *Phylloscopus collybita*, *Turdus merula*, *Parus major*, *Dendrocopus major*, and

Fringilla coelebs. Notably, a nesting site of *Bubo scandiacus*, commonly known as the snowy owl, can be found along the educational trail (Kelemen, 1978), making it a significant spot for bird enthusiasts.
The western part of the area mainly consists of agricultural fields, which may not be the ideal location for a pleasant walk. Against this, the eastern region is an exceptional exhibit of biodiversity and serves as a natural ecological museum. Thus, it is expected that this area will be the primary educational focus of the trail, providing a captivating and informative learning experience. Therefore, the analysis results support the decision to exclude the western hill section and focus on planning the eastern portion instead.

3. Analysis of the central area - Churchyard

As observed in the analysis of the area, the functions are distributed without coherence between them. The secondary road, which intersects the two spaces, appears redundant due to its rare usage. Based on traffic analysis, the elimination of motorized traffic from streets can create a coherent connection between the space and functions. By implementing modifications and removing trees, a structural configuration and sense of space can be established, with axes being introduced. In summary, it is imperative to tackle the primary issue of the road section that separates the two areas.
The concepts of memorial square and church can be intertwined, resulting in a community-oriented space that fosters a sense of history and devotion. Furthermore, it may be necessary to prohibit parking in the proximity of the square during church services to prevent congestion caused by parked cars.
Promoting tourism is a favorable opportunity for the settlement, considering its abundant vegetation, natural attractions, and historical monuments, landmarks.

CONCEPT

The scheduling of the plan will follow a similar approach to the analyses, proceeding from larger to smaller scales. Firstly, the concept for the educational trail will be discussed, followed by the plan for the churchyard.

The design of the ecological learning trail will be based on the analyzed principles. This involves mapping the key points discovered during the research, including old trees, flora and fauna, rest areas, and lookout points, and categorizing them accordingly. The second component will consist of a network of nature trails based on these categories, which will be constructed by extending existing field roads or by adding partial extensions to them (Figure 8).

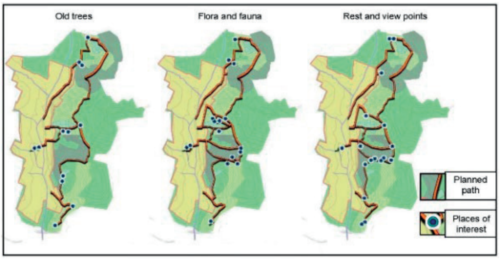

Figure 8. The concept of the ecological trail (according to: General urban plan of Pănet, 1998)

The organizational concept for the churchyard and central part of the site is based on the principles of site planning, which involve creating a cohesive plan that organizes the site according to the structure of its included axes and built elements. The concept will be based on three primary axes, as illustrated in Figure 9. The plan aims to preserve and expand upon the basic functions of the old yard and its surroundings while reflecting the principles formulated in the analyses, such as maintaining a traditional character and serving as the center of community life.

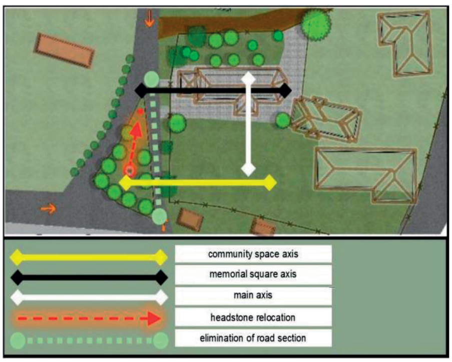

Figure 9. The concept of central area

PILOT PROJECT, INTERVENTIONS

1. Ecological educational trail

In the ecological learning trail, the identified points are categorized and mapped to establish a network of the ecological nature trail. Following the elimination of any overlaps, the resultant trail is approximately 2 kilometers in length, consisting of a primary circuit route complemented by two optional, more challenging sections (Figure 10). These sections comprise steep inclines and incorporate two stations inaccessible via any other route. The ultimate trail consists of a total of twenty-one (21) stations.

The recommended starting point for the ecological learning trail's planned area unit is at the northernmost point. However, in the absence of a designated guide, hikers can also begin at two other points. One of these points originates from the churchyard, and the hiker would enter the middle section of the trail. In the southern part, the hike would commence with a steep incline, leading to the first station after a relatively long climb. These two road extensions are included because they intersect with two bus stops and two cafes, where hikers can take a rest during a specific section of the trail. Upon returning to the starting point, hikers will encounter a vertical information board displaying the road network of the educational trail, its difficulty level, and general information about the nearest station points and noteworthy features. The board's style adheres to tradition and features a motif that appears in some part of all furniture types included in the plan, thus creating a cohesive look for the learning trail and the central space separately. The visual aspect is prioritized, and a specially designed lookout point will be constructed at the viewpoint areas. The ecological learning trail is designed to blend in with the natural environment, requiring minimal landscaping. Rest areas are available at each station, with log benches that comfortably accommodate two people, providing an immersive nature experience.

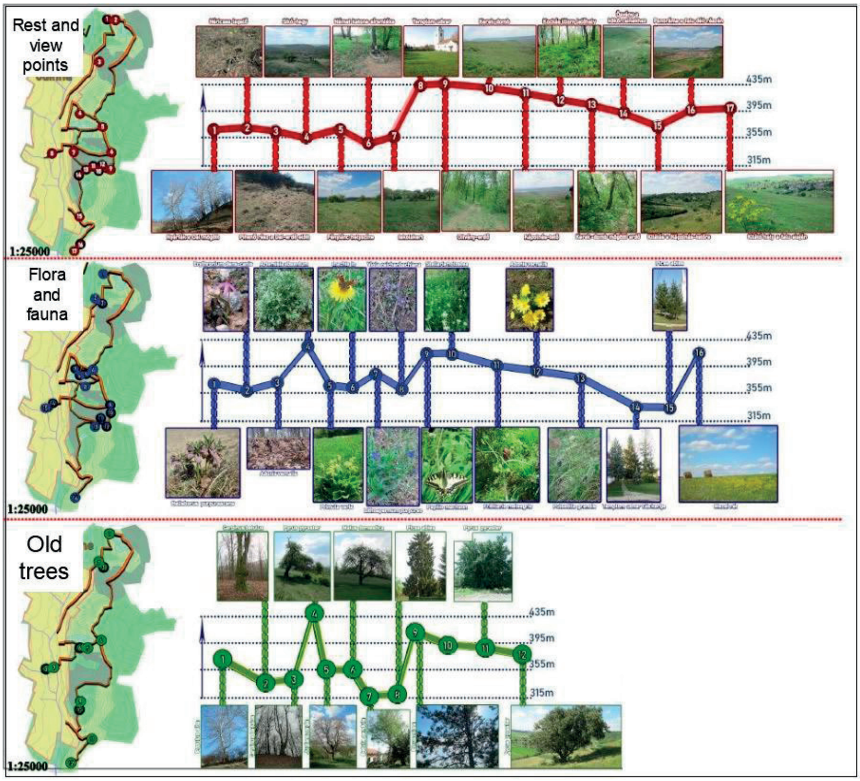

Figure 10. Masterplan and section of the proposed ecological trail (according to: General urban plan of Pănet, 1998)

The stations dedicated to rare and characteristic plant sites were developed with strict parameters to minimize trampling and contact with the plants. It is crucial to preserve the existing vegetation and minimize human impact on the natural ecosystem. Thus, it is not recommended to introduce new plants into the area of the ecological learning trail.

2. Historical perspective, the churchyard

The churchyard represents a unique planning program that is closely tied to the ecological trail. This connection is not only evident in its role as a station but also in its furniture and built elements. The underlying organizational principle aims to establish three functionally distinct axes that can merge to create a coherent and integrated entirety.

Figure 11. Perspectives from the proposed churchyard design

The planned area units of the churchyard aim to integrate the memorial square and the church yard by removing an underutilized section of the road and expanding the green area. As a result, the surface area will increase, which remains functional while being separated by the church's fence. The newly constructed Szekeler gate, previously without a clear purpose, will function as an entrance from the public square to the churchyard, making effective use of its design.

Six (6) *Quercus robur*, commonly known as oak trees, were deliberately planted in a row to offer uninterrupted vistas of the church from the western and southern approaches (Figure 11). This tree species is indigenous to the local countryside, thus representing the natural environment and ethos of the ecological learning trail.

The surface material used in the pedestrian areas surrounding the church and public square consists of precisely cut granite cubes with dimensions of 5 x 5 x 5 cm. The same split stone material is used for the detour path that passes through the pergola, creating a consistent visual appearance throughout the area.

The proposed vegetation design exclusively incorporates hygrophyte plants due to the elevated groundwater level in the area. The following Table 1 list the species along with their respective codes and quantities.

Table 1. Proposed vegetation

ID	Scientific name	Common name	Quantity
1	*Quercus robur*	penduncle oak	6
2	*Cornus sanguinea*	dogwood	7
3	*Spirea* x *vanhouttei*	bridalwreath	20
4	*Crataegus laevigata*	woodland hawthorn	6
5	*Prunus domestica* 'Stanley'	European plum	3
6	*Prunus domestica* 'Ruth Gerstetter'	European plum	2

CONCLUSIONS

The educational trail highlights the values of the past and presents them in a modern way that can be approached from multiple perspectives. It establishes a symbolic linkage that connects the present natural areas with the past central areas and the church, thereby bridging the gap between the two temporal domains.

The plan incorporates goals and principles that reflect the village's image, traditions, and modern spatial structure. The modern spatial structure complements rather than subjugates these values.

The ecological learning trail presents a valuable opportunity to showcase the wonders of nature in an engaging and informative way. A walking time of approximately 2 hours, including rest and lookout areas, is optimal for a fulfilling experience.

The plan was developed with a deep appreciation for nature, avoiding any attempt to subjugate it. Considerations such as appropriate paving materials, road network design, information board size, and furniture shape all contribute to a sense of connection with nature. This educational tool is expected to enable hikers to form a deeper connection with nature, appreciating its beauty and precious mystery, and developing a sense of responsibility to protect it.

The central part of the village serves as the anchor point for the planning design, with faith, community, and memory being the fundamental pillars of the society. The plan effectively integrates these pillars by creating communal green space, the churchyard, and the pergola, which encourage reflection, as well as the monument, all of which are integral elements of the village's identity. Additionally, due to the strategic placement of one of the educational trail's stations, the plan creates a cohesive and valuable unity for Pănet.

Ecotourism can provide "close-to-nature" areas for both residents and visitors, offering opportunities for recreation and relaxation, which are increasingly important in the context of expanding cities and overcrowding. As people's demand and need for such areas continue to grow, ecotourism can play a crucial role in meeting these needs while also promoting sustainable practices and preserving natural environments.

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GROWTH AND FLOWERING PERFORMANCE EVALUATION OF TEN POTTED *CHRYSANTHEMUM* CULTIVARS

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Abstract

Chrysanthemum is one the most important flower crops commercially grown for cut flower, loose flowers, bedding and pot plants. Along with ensuring optimal culture conditions, an appropriate nutrition regime and the application of appropriate management, the most important factors on which the success of a chrysanthemum containerized culture depends are: the choice of suitable varieties for containerized culture, high-quality planting material. In order to diversify the current Chrysanthemum assortment and to promote of the most valuable cultivars for containerized culture, an experiment was conducted to evaluate the growth and flowering performance of ten Chrysanthemum cultivars, in greenhouse conditions. From the data presented, taking into account both flowering phenology and morphological characters, decorative features, the best varieties within the studied Chrysanthemum assortment were Meridian White and Sumbeam Pink Bicolor.

Key words*: Chrysanthemum, cultivars, flowering, growth, potted plants.*

INTRODUCTION

Chrysanthemum is the most important flower crop in the Asteraceae family, a multiple-use plant that has a market value ranking second highest among ornamental species following the rose (Shinoyama et al., 2012).

Chrysanthemums are used either as cut flowers or grown in pots and the success in cultivation of this plant is principally due to the great diversity of species and cultivars, suitability in different cultivation systems and the possibility of cultivation all over the year (Uddin et al., 2015).

Chrysanthemum morifolium Ramat. Tzvel, the most remarkable taxon of *Chrysanthemum*, originated in China, is a medicinal, food and ornamental plant (Hao, 2022).

As an ornamental plant, it is found in the most diverse forms, with flowers that cover almost the entire range of known colors with the most varied sizes and shapes, from single to double, wrapped, stellate, globular.

The erect and tall growing types (standard chrysanthemums) are grown as cut flowers for making bouquets and vase decoration the dwarf and compact growing types (spray chrysanthemums) are cultivated as pot plants for beautifying indoors and outdoors spaces (Singh & Chettri, 2013).

Advancement in pot *Chrysanthemum* varietal development and production technology permits its year-round production in most parts of the world, in greenhouses by managing environmental conditions. Along with ensuring optimal environmental conditions, an appropriate nutrition regime and the application of appropriate management, the most important factors on which the success of a chrysanthemum container culture depends are: the choice of suitable varieties for container culture, high-quality planting material.

Today, more and more private producers are focusing on growing chrysanthemums in pots to be used in the fall. In this regard, the preference of varieties will be provided according to the period in which it is desired to obtain pots with well-developed and flowering plants to have a commercial appearance (Cantor et al., 2020). A potted chrysanthemum can be considered good when it has a good shape, compact appearance, relatively low height, abundant and long-lasting flowering. For choosing the good cultivars, varietal performance has previously evaluated on different crops like *Gerbera* (Petra et al., 2020), *Gladiolus* (Azimi, 2020, Nicu & Manda, 2022, Swaroop et al., 2022), *Tagetes* (Narsude et al., 2010, Shivakumar et al., 2015), *Lisianthus* (Hasib et al, 2017), *Hemerocallis* (Bahrim et al., 2020), *Hosta* (Aelenei et al., 2020) and all of these crops showed variations in their performances. Many studies for this purpose have also been carried out on *Chrysanthemum* (Roude, 1991, Bala, 2015, Uddin et al., 2015 Suvija et al., 2016, Parmar et al., 2019, Cantor, 2020, Patil et al., 2022, Prakash & Fatmi 2022, Priya & Singh 2022, Singh et al., 2022).

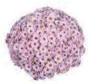
In Romania, nowadays, the cultivated areas with *Chrysanthemum* are developing quickly through potted cultures which have gained a significant increase. Also, due to the progress made in the selection of chrysanthemums, many cultivars have been introduced in the recent plant assortment. The knowledge of the morphology and biology of the new cultivars is very important because can be recommended varieties that will be adequate to the Romanian local conditions (Cantor, 2020). In order to diversify the current assortment and to promote of the most valuable cultivars for containerized culture, an experiment was conducted to evaluate the growth and flowering performance of ten *Chrysanthemum* cultivars, in greenhouse conditions.

MATERIALS AND METHODS

The study was carried out in 2020, in Daneti township, Dolj county, in a chrysanthemum protected culture, established on 01.06.2020, in a solar with a surface of 500 m, under natural light conditions. The climate is temperate-continental in Daneti and it is characterized by very hot summers, with low rainfall and moderate winters. In 2020, the average annual temperature was 13.6°C and the amount of annual rainfall was 690.1 mm.

Plant material was represented by ten medium vigor *C. morifolium* Ramat. cultivars: Daybreak Sweet Pink (DSP), Daybreak Purple (DP), Daybreak Bright Yellow (DBY), Meridian White (MW), Meridian Bronze (MB), Sunbeam Flame (SF), Sunbeam Pink Bicolor (SPB), Sunbeam Coral (SC), Chrystal Misty Purple (CMP), Gigi Snow (GS) (table 1).

Table 1. Description of of *Chrysanthemum* varieties studied

Daybreak sweet pink	**Daybreak purple**	**Daybreak bright yellow**	**Sunbeam flame**	**Sunbeam pink bicolor**	**Sunbeam coral**	**Meridian white**	**Meridian bronze**	**Chrystal misty purple**	**Gigi snow**
									
Flowering time									
very early	very early	very early	early	early	early	mid season	mid season	mid season	Mid season
Flower color									
pink	purple	yellow	bronze golden	pink bicolor	red	white	bronze	pink	white
Plant vigor									
medium	medium	medium	medium	medium	medium	medium	medium	medium	medium

Cuttings purchased from import (certified planting material) on 27.04.2020, from Dummen Orange Group, were used to establish the culture. These were planted for rooting in jiffy pills. The percentage of rooting was assessed two weeks after the establishment of cultures and was 100%.

After rooting, the cuttings were planted in 10.5 cm pots and a phosphorus-based fertilizer was applied. After three weeks, the first pinch was performed at 4-5 leaves from the surface of the substrate. After another 2 weeks, the first treatment with growth retardants (B-Nine 85, 0.3%) was applied, and the second at the appearance of flower buds. After 20-25 days (01.06.2020), when they exceeded the diameter of the pot, the plants were transplanted into the culture pots (18 cm diameter), in a mixture of peat + perlite (3:1) in which Osmocote was incorporated and the drip system was installed.

In the first month of cultivation, the green Universol fertilizer was used, and after the plants exceeded the diameter of the pot, the blue Universol 18+11+18 was introduced, for about 40 days until the appearance of the first flower buds. Two Universol yellow 12+30+12 and Peters fertigations were applied at the appearance of buds and after the appearance of

flower buds, Universol base was used until the completion of the culture. All other cultural practices were kept uniform for all treatments in the experiment. Throughout the vegetation period, the main phenophases were followed: the beginning of flowering, the mass flowering, the end of flowering. Based on the phenology data, the following were calculated: the number of days from the establishment of the culture to the beginning of flowering, the duration of the flowering, the number of days from the establishment of the culture to marketing stage.
The morphological and ornamental characteristics determined were: plant height, plant diameter, number of shoots, internode length, number of leaves/shoot, leaf length, number of flowers/shoot, flower head diameter. The plant height, internode length, and number of leaves was recorded at flowering stage. Due to excessive growth, the cultivars Chrystal Misty Purple and Gigi Snow were removed from the study, as they lost their decorative appearance.
The experiment was laid out in Completely Randomized Design with three replications. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and means were separated using the Tukey's pairwise comparisons at a significance level of $p \leq 0.05$ using the MINITAB package system.

RESULTS AND DISCUSSIONS

Considering the commercial importance of this crop, there is a necessary to evaluate cultivars for their vegetative, flowering and quality characters for the purpose of identification of suitable cultivars for specific regions.
The study was conducted to evaluate the performance of some *Chrysanthemum* cultivars in respect to their different traits.
Analyzing Table 2, it follows that the beginning of flowering was triggered the earliest in the varieties Daybreak Sweet Pink, Meridian White, Sunbeam Flame, 64-66 days after the establishment of the culture, followed by the varieties Sunbeam Pink Bicolor, Daybreak Purple which bloomed after 71-75 days, and the varieties Daybreak Bright Yellow, Meridian Bronze, Sunbeam Coral bloomed after 77-81 days from the establishment of the culture.
Also, there is observed a staggered flowering within the analyzed assortment, which includes varieties with early (Daybreak Sweet Pink, Daybreak Bright Yellow, Sunbeam Flame), medium (Daybreak Purple, Sunbeam Pink Bicolor, Sunbeam Coral) and late flowering (Meridian White, Meridian Bronze) (Table 2).

Table 2. The main phenophases of *Chrysanthemum* varieties studied

Cultivar	Flowering			Marketing stage	Days to beginning of flowering	Duration of flowering (days)	Time to marketability (days)
	Beginning of flowering	Full flowering	End of flowering				
DSP	03.08	20.09	08.10	20.08	64	66	81
DP	14.08	27.09	10.10	17.08	75	56	78
DBY	04.08	20.09	01.10	12.08	65	58	73
SF	05.08	12.09	04.10	20.08	66	60	81
SPB	10.08	17.09	12.10	12.08	71	63	73
SC	15.08	23.09	10.10	20.08	78	59	81
MW	20.08	24.09	13.10	25.08	81	64	86
MB	16.08	21.09	16.10	20.08	77	61	81
Mean	03-20.08	12-27.09	01-13.10	12-25.08	72.12	60.87	79.25
SE	-	-	-	-	1.82	0.85	1.20
SD	-	-	-	-	6.04	2.83	3.99

*DSP-Daybreak Sweet Pink, DP-Daybreak Purple, DBY-Daybreak Bright Yellow, SF-Sunbeam Flame, SPB-Sunbeam Pink Bicolor, SC-Sunbeam Coral, MW-Meridian White, MB-Meridian Bronze.

The longest duration of flowering was recorded in Daybreak Sweet Pink (66 days) followed by Meridian White and Sunbeam Pink Bicolor (64 days, 63 days respectively) and the shortest flowering period corresponds to the Daybreak Purple cultivar (56 days). Compared to the average of all the varieties (60, 87 days), lower values were obtained for the Daybreak Purple, Daybreak Bright Yellow, Sunbeam Flame and Sunbeam Coral (56-60 days). The difference in the flowering duration may be due to the varietal character, environmental factors, habitat type and genetic makeup of the varieties. Similar results were reported in *Chrysanthemum* by Balaji & Reddy (2004) and Srilatha et al. (2015).
The number of days from establishment to marketable stage was between 73 days

(Daybreak Bright Yellow, Sunbeam Pink Bicolor) and 86 days (Meridian White). Intermediate values were recorded at Daybreak Purple (78 days) and at Daybreak Sweet Pink, Meridian Bronze, Sunbeam Flame and Sunbeam Coral (81 days) (Table 2).

The observations and the measurements achieved concerning the main morphological characteristics of *Chrysanthemum* cultivars studied are presented in the Table 3. Plant height presented statistically significant variation, the range of plant height was from 50.60 cm to 58.63 cm. The tallest plant was found from Sunbeam Flame (58.63 cm) followed by Sunbeam Coral (58.17 cm), whereas Daybreak Purple, recorded the lowest plant height of 50.6 cm. All the cultivars exceeded the criterion for acceptable plant height established for potted plants, which is from 1.5 to 2.5 times pot height (Sitawati & Ni'mah, 2021). Moreover, the varieties Chrystal misty purple and Gigi snow were eliminated from the study, because they lost their decorative appearance due to exaggerated vegetative growth. As a result, cuts were applied followed by additional treatments with growth retarders, which no longer allowed their comparison with the other varieties studied. As mentioned by Sitawati & Ni'mah (2021), depending on the growth rate of chrysanthemum varieties, the number and frequency of treatments with daminozide is different: fast-growing cultivars required daminozide application three times, moderate-growing cultivars required once daminozide application, and slow-growing cultivars does not require the application of PGR to produce a chrysanthemum height to the suitable size standard for potted plants. Therefore, further studies are needed to investigate the effects of PGR application on chrysanthemum cultivars grown in pots. Qureshi et al. (2018) mention that 2 applications at four weeks after planting and at flower initiation with 8000 ppm daminozide, does not meet the quality standard for several types of potted chrysanthemums; a single application of daminozide did not produce significant results, but the second and subsequent applications may reduce plant height. It could be concluded that it is necessary to more study the effect of PGR application on chrysanthemum cultivars grown in pots.

The results revealed that the highest plant diameter was recorded in Sunbeam Flame (64.46 cm), followed by Sunbeam Coral (60.05 cm) and Meridian white (60.00 cm), while the Sunbeam Pink Bicolor have registered the smallest increases in the diameter of the plant of 55.76 cm. The average diameter of the plant in Sunbeam Flame was significantly higher compared to all the other varieties analyzed.

No significant differences were found between cultivars regarding the number of branches per plant which ranged from 4 branches/pl (Daybreak Purple, Sunbeam Pink Bicolor) and 4.83 branches/pl in the Sunbeam Flame cultivar. Intermediate values were recorded for other varieties, ranging from 4.20-4.67 branches/pl. These results were confirmed by Meilasari et al. (2021) who showed that by pinching the apical buds in chrysanthemum cultivars grown in pots, 3-4 lateral shoots are expected to grow.

The length of the internode varied between 2.83 cm in the Sumbeam Pink Bicolor variety and 3.43 cm in the Daybreak Bright Yellow variety, with other varieties recording intermediate values between 3.1-3.2 cm. Compared to the average of all the varieties (3.15 cm), the Daybreak Sweet Pink, Daybreak Purple, Daybreak Bright Yellow, and Sunbeam Pink Bicolor varieties had lower values ranging from 2.83-3.13 cm. The lack of correlation between plant height and internode length is explained by the difference in the number of nodes (Table 3).

The average number of leaves per shoot and the average leaf size are also important parameters for evaluating container-grown chrysanthemum varieties. A large number of leaves per plant is reflected in the compact appearance and commercial value of the variety. There were significant differences between cultivars for average number of leaves per shoot. The highest value of this parameter corresponds to the Sunbeam Flame variety (83 leaves), followed by Meridian White (81.3 leaves), and the lowest value was recorded for the Daybreak Purple variety (58.3 leaves), which also had the lowest height. Compared to the average of the varieties (73.07), lower values were obtained for the Daybreak Purple and Meridian Bronze varieties.

In terms of the average leaf length, the analysis of the data shows that the highest value was recorded at the Meridian White (5.23 cm), followed by Daybreak Sweet Pink (5.20 cm) while the lowest value was recorded for the Daybreak Purple variety (4.53 cm). The average leaf length in Meridian White was significantly higher compared to Daybreak Purple cultivar. Compared to the average of all the varieties (4.91 cm), lower values were obtained for the varieties Daybreak Purple, Sunbeam Coral, Sunbeam flame and Meridian Bronze (4.53 cm, 4.67 cm, 4.78 cm, 4.90 cm respectively).

Table 3. Performance of *Chrysanthemum* cultivars for morphological characters

Cultivar	Plant height (cm)	Plant Diameter (cm)	Number of branches/pl	Internode length (cm)	Number of leaves/shoot	Leaf length (cm)
DSP	52.00±6.03 ab	57.76±1.36 bc	4.56±0.40 a	3.10±0.10 ab	75.20±2.43 b	5.20±0.26 ab
DP	50.60±1.68 b	56.53±0.98 c	4.00±0.23 a	3.03±0.20 ab	58.33±1.52 c	4.53±0.30 b
DBY	52.86±1.80 ab	58.33±1.53 bc	4.20±0.34 a	3.13±0.12 ab	76.00±9.29 a	5.00±0.20 ab
SF	58.63±1.52 a	64.46±1.28 a	4.83±0.28 a	3.43±0.11 a	83.00±3.00 a	4.78±0.25 ab
SPB	54.70±2.52 ab	55.76±0.68 c	4.00±1.00 a	2.83±0.15 b	72.73±1.42 b	5.00±0.20 ab
SC	58.17±1.76 a	60.05±1.32 b	4.50±0.50 a	3.27±0.11 a	74.60±5.03 b	4.67±0.30 ab
MW	55.20±0.80 ab	60.00±1.00 b	4.67±0.58 a	3.17±0.15 a	81.46±1.86 a	5.23±0.12 a
MB	55.86±1.20 ab	58.67±1.15 bc	4.33±0.58 a	3.27±0.15 ab	63.57±3.11 bc	4.90±0.26 ab
Mean	54.79	59.04	4.49	3.15	73.07	4.91
SE	0.71	0.57	0.11	0.042	1.78	0.06
CD	3.46	2.78	0.53	0.20	8.75	0.31

* Means within rows using different letters are differ significantly at the P value ≤ 0.05 levels; **DSP-Daybreak Sweet Pink, DP-Daybreak Purple, DBY-Daybreak Bright Yellow, SF-Sunbeam Flame, SPB-Sunbeam Pink Bicolor, SC-Sunbeam Coral, MW-Meridian White, MB-Meridian Bronze.

The average number of flowers per shoot was determined at the time of maximum flowering and includes both open flowers and buds formed. The values for this parameter varied within a wide range, ranging from 29 flowers/shoot for Sunbeam Coral to 48.3 flowers/shoot for the Meridian White variety, the difference being statistically ensured (Table 4).

Table 4. Performance of *Chrysanthemum* cultivars for flowering parameters

Cultivar	Number of flowers/shoot	Diameter of heads (cm)
DSP	45.30±2.52 ab	5.30±0.26 a
DP	38.60±1.51 b	4.87±0.30 ab
DBY	41.50±2.18 ab	5.03±0.25 ab
SF	38.90±3.76 b	5.10±0.36 ab
SPB	44.40±4.06 ab	4.90±0.36 ab
SC	29.00±1.50 c	4.60±0.40 ab
MW	48.50±1.50 a	5.10±0.43 ab
MB	43.26±2.97 ab	4.30±0.25 b
Mean	42.42	4.89
SE	0.83	0.08
SD	4.07	0.43

*Means within rows using different letters are differ significantly at the P value ≤ 0.05 levels (using one way analysis)

Compared to the average of all the varieties (4.91 cm), lower values were obtained for the Daybreak Purple, Sunbeam Coral, Sunbeam flame and Meridian Bronze varieties (4.53 cm, 4.67 cm, 4.78 cm and 4.90 cm respectively).

The average diameter of head recorded the highest value in Daybreak Sweet Pink (5.3 cm), followed by the Sunbeam Flame and Meridian White (5.1 cm), and the lowest value it was recorded for the Meridian Bronze variety (4.3 cm). Compared to the average of all the varieties (4.89 cm), lower values were obtained for the Daybreak Purple, Sunbeam Coral and Meridian Bronze (4.3-4.87 cm).

CONCLUSIONS

Based on the observations it can be concluded that in container-grown chrysanthemum cultivars, shorter plant height, shorter internodes, and a higher number of heads per plant can be advantageous for vegetative growth. The different cultivars exhibited significant variations in most parameters. No significant differences were observed in the number of shoots per plant. All the cultivars exceeded the plant height criterion established for potted plants. The Daybreak Sweet Pink, Meridian White, and Sunbeam Pink Bicolor cultivars had the longest flowering duration, while the Daybreak Purple cultivar had the shortest. The Meridian White and Sunbeam Pink Bicolor varieties performed best in most parameters, including plant size, early flowering, average number of heads per plant, and duration of flowering, followed by the Daybreak Sweet Pink cultivar.

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BIRCH SAP HARVESTING IN CHANGING SPRING CONDITIONS AND ITS IMPACT ON TREES GROWTH

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Abstract

The collection of birch sap in spring has become, in the past decades, a regular practice in Romania, because of its multiple health benefits. Over the years, many collectors experienced unsatisfactory results in terms of the amount of sap harvested, usually attributed to the unpredictable spring weather of some years, with large variations from a day to another. The results of this study revealed that independently of weather conditions in spring, the best period of sap harvesting in North-East of Romania was between 25th of March - 5th of April, when the air temperatures did not exceed 15^{o}C. Trees higher than 20 m were most productive. At the end of the growing season, tapped trees were smaller than those untapped. These results suggest that in time, the tapped trees are less productive due to loss of vigour rather than spring weather conditions.

Key words: *Betula pendula, climatic changes, sap production, trees height.*

INTRODUCTION

European countries are affected by global warming, which has a significant impact on the climate (Anders et al., 2014). Seasons are frequently abnormal in terms of both temperature and precipitation' regimes, with implications on vegetation (Sukopp & Wurzel, 2003; Gloning et al., 2013; Loupian et al., 2017; Liu et al., 2020). Climatic changes affect woody plants communities all around in Europe. Various effects were observed, from forest scale, such as distribution of species (Heuertz et al., 2010; Czúcz et al., 2011; Silva et al., 2012; Matías & Jump, 2014) and biodiversity/composition (Ruiz-Labourdette et al., 2013; Morin et al., 2018; Vacek et al., 2021) to individuals, for instance, species interactions (Kleinbauer et al., 2010; González-Muñoz et al., 2014), drought-induced dieback (Martinez-Vilalta et al., 2012), and changes in growth (Vieira et al., 2020; Zhirnova et al., 2021). Climate change have an essential impact on the non-wood forest products (Kirilenko & Sedjo, 2007; Murphy et al., 2012; Gurung et al., 2021), because their quantities and qualities depend on the ability of trees to manage with the new conditions. In case of silver birch (*Betula pendula*), which is a common species in Europe, it revealed an excellent acclimation capacity to light and temperature (Rousi et al., 2012). However, although the outset of spring bud burst is controlled by photoperiod and heat sum (Hänninen & Tanino, 2011; Hawkins & Dhar, 2012), birch trees' survival and adaptation to climate changes seems to be dependent by nitrogen soil availability (Possen et al., 2021).

In Romania, the collection of birch sap in spring has become, in the past decades, a regular practice, mainly because of its benefits on different organs such as kidneys, liver, lungs, stomach or skin (Svanberg et al., 2012; Papp et al., 2014). Birch sap can be harvested from both rural and urban trees, without but it may have a different composition when those experience environmental stress (Grabek-Lejko et al., 2017).

The birch sap harvest begins in March and ends in May, before the foliage state (Papp et al., 2014). Over the years, in Romania, many collectors experienced unsatisfactory results in terms of the amount of sap harvested, usually attributed to the unpredictable spring weather of some years, with large variations from a day to another. Therefore, the present research identifies the ability of birch trees to deal with climate change in terms of sap production and the impact of this practice on trees growth.

MATERIALS AND METHODS

Investigations were carried in 2020 and 2021, on twenty silver birch trees (*Betula pendula* Roth.) growing in a forest near Râşca village (47°21′25″N, 26°14′4″E, 399 m altitude), Suceava County, România. Birches grow here on a podzolic soil of normal humidity.

During the two years of studies, the weather conditions in the region were quite different. Thus, the mild winter of 2019/2020 was followed by a spring with a cold start, recording 13 days with minimum negative temperatures in March and eight days in April. Also, during March and April, warm days were frequently followed by intense cooling. At the end of March, the last snowfall covered the soil with 8 cm of snow and after that, April was completely dry. In contrast, winter 2020/2021 was colder, with frequent episodes of frost and snow. First part of spring was also cold, with 17 days of minimum negative temperatures in March and the last snowfall was recorded in the second decade of the same month. After that, spring was warm and relatively dry.

Birch sap was harvested from the same marked trees during March-April of the two years. Trees were tapped on 10th March, prior to the start of sap flow. In each trunk at a height of 50 cm above soil surface, a single hole was open in the same position on each tree, with the diameter of 10 mm and 3-4 cm depth, using a drill. Then a plastic tube was inserted into the hole and the other end attached to a plastic container. Sap was collected and measured for each individual, daily.

Growth of the tapped trees was evaluated by measuring their height, using a Haglöf Vertex 5 Hypsometer and diameter at breast height (dbh), using a tree caliper. Data were recorded in March, before extracting the sap, and at the end of growing season, in November. For comparison, the same measurements were made simultaneously for non-tapped birch trees. Also, the age of every tree was determined, using a Pressler borer.

Statistical analysis were applied to evaluate the influence of trees parameters (tree height, crown height, diameter at breast height and age) on the sap production, using linear regression and Pearson test. The impact of sap harvest on trees growth was analysed using one-way ANOVA test, which was applied on the data representing the differences among height and dbh of both tapped and untapped trees, before and after growing season, then statistical differences between means were estimated with the Least Significant Difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSIONS

Sap started to flow in the second decade of March, independent of spring weather conditions in 2020 and 2021(Figure 1). The highest average amount of sap was harvested between 25th of March and 5th of April, representing 65% of the total sap collected. Even if in 2020, a few warm days in March initiated the sap flow a week earlier (on 19th March), the sudden cooling that followed, stopped it.

The sap harvesting takes place in almost same period also in some other European countries such as Denmark (Sancho et al., 2022), Germany (Westhoff et al., 2008), Finland (Harju and Huldén, 1990), Lithuania (Mingaila et al., 2020), Latvia (Kūka et al., 2013), Poland (Zajączkowska et al., 2019; Staniszewski et al., 2020), and Ukraine (Zyryanova et al., 2010). Although Europe has a varied climate, continental weather experienced a similar warming trend in recent years (Van Heerwaarden et al., 2021). Therefore, higher temperatures in Northern Europe accelerated the springtime phenological evens of birch trees (Emberlin et al., 2002; Ahas et al., 2002; Olsson & Jönsson, 2014; Minin et al., 2016) and sap can be collected around the same period as in Eastern Europe.

Values of air temperature at the time of harvesting had an important impact on the sap flow rate. At negative temperatures during day, complete cessation of sap flow was observed for all trees.

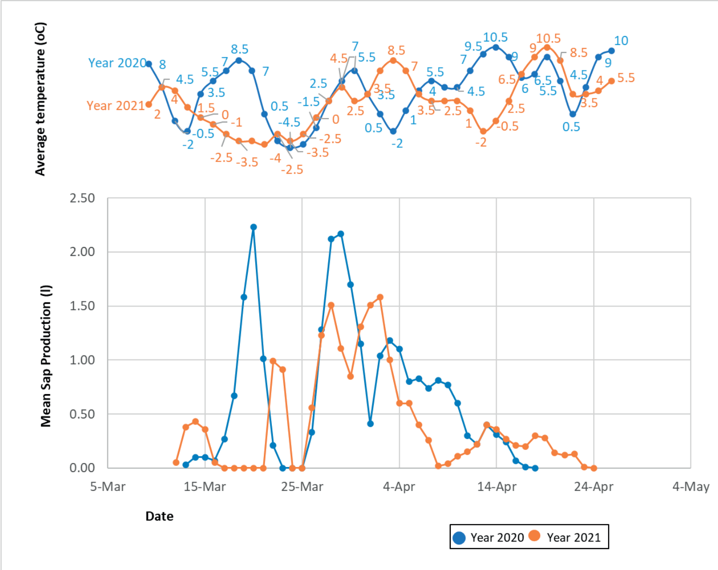

Figure 1. Mean quantity of sap (l) collected from silver birch trees, during the spring of 2020 and 2021

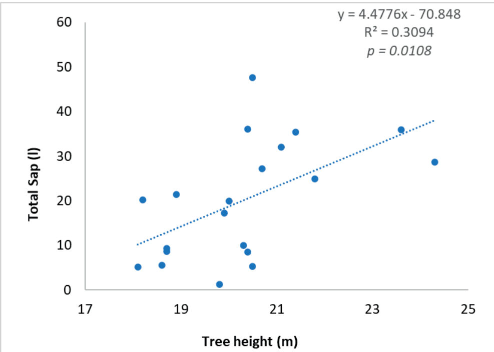

Figure 2. Positive correlation between total sap and tree height (significant at $p < 0.05$)

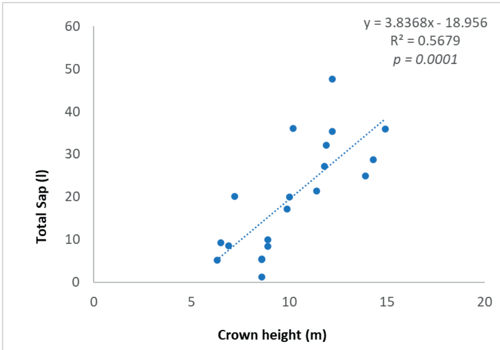

Figure 3. Strong correlation between total sap and crown height (significant at $p < 0.01$)

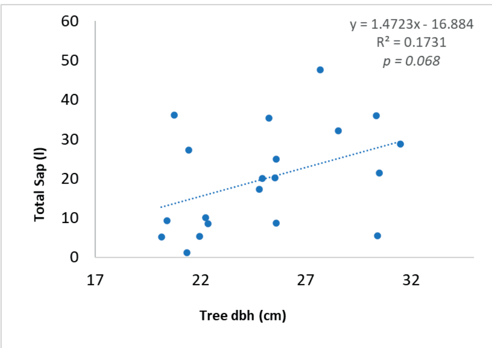

Figure 4. Relationship between total sap and diameter at breast height (dbh)

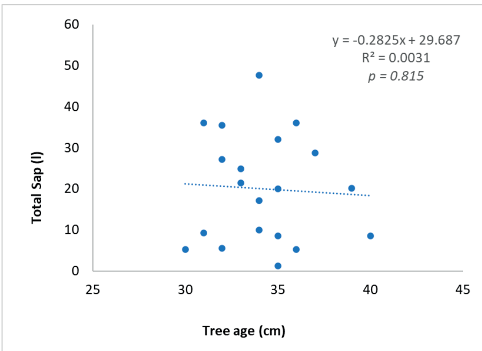

Figure 5. Relationship between total sap and tree age

Also, sap flow decreased when maximum temperatures exceeded 10ºC. The relationship between sap flow and air temperature was reported by other researchers (Harju and Huldén, 1990; Westhoff et al., 2008; Hölttä et al, 2018).

Low precipitation rate or drought do not seem to affect sap flow in birches (Gartner et al., 2009; Baumgarten et al., 2019; Sullivan et al., 2021). Still, silver birch trees (*Betula pendula*) are sensitive to prolonged drought (Beck et al., 2016; Dox et al., 2022).

The exudation period lasted 35 days in spring of 2020 and 43 days in 2021. Birch trees exude even longer, up to 47 days in northern Europe (Essiamah, 1980). This variation depends on temperature.

Although the sap flow lasted longer in the spring of 2021, the total quantity of sap was not greater than in 2020.

Total quantity of sap harvested from each silver birch was positively correlated (Figure 2) with tree height ($r = 0.5562$; $r^2 = 0.3094$; $p = 0.0108$). Thus, much more sap was collected from trees taller than 20 m. Moreover, sap quantity was strong positive correlated with crowns height (Figure 3).

The highest quantity of sap was harvested during spring of 2020, totalling 47.7 liters, from a tree of 21 m in height and with a crown height of 12 m. From the same tree was collected the highest quantity of sap as well in the following year.

For most of the investigated birches, the amount of harvested sap was lower in 2021. Thus, the total sap production in the spring of 2021 represented only 80% of that of 2020. A decrease in sap production of trees exploited in consecutive years has also been reported by Maher (2005).

No significant relationship was found between total sap harvested and dbh or tree age (Figures 4 and 5).

Some authors remarked a corelation between sap quantity and dbh (Shi et al., 2001; Maher, 2005; Mingaila et al., 2020) and other, did not (Ganns et al., 1982; van den Berg et al., 2013). Generally, dbh is one of the most used parameters that guide collectors to select trees for tapping, but sap yield proved to be more in relation with several other factors, such as birch species (Zyryanova et al., 2010), position of tree in the forest stand (Zajączkowska et al., 2019) or soil type (Mangaila et al., 2020). Anyway, the cardinal location of the boreholes in the tree trunk does not affect the sap quantity (Kopeć et al., 2020).

Sap harvesting in the spring of 2020 and 2021 had a negative impact on trees growth. Trees height were significant reduced comparative with untapped trees (Table 1) at the end of growing season.

Table 1. Comparative growth parameters at the end of 2020 and 2021 at untapped and tapped trees

Parameters	Untapped Trees		Tapped Trees		LSD
	2020	2021	2020	2021	
Average height differences (m)	0.61	0.43	0.39*	0.26*	*0.16*
Average dbh differences (cm)	0.38	0.60	0.33	0.39	*0.23*

LSD – Least Significant Difference

Asterix indicates a statistically significant difference (*P*<0.05, LSD test) from untapped trees.

Although the values of dbh were also smaller at tapped trees, these were not found statistically significant at $P<0.05$.

Springtime phenological events relies on xylem sap. In both years of studies, sap harvesting delayed the budburst. Moreover, drought restrict bud development and growth (Kukk et al., 2015). Consequently, although birches have indeterminate growth habit (Hara et al., 1991; Pothier & Margolis, 1991; Weih, 2000; Zarnovican, 2000), shoots extension period was shorter in tapped birch trees, and recorded lower heights in autumn. Our findings are confirmed by Rousi and Pusenius (2005), which concluded that the best predictor of silver birch growth is length of the growing period.

CONCLUSIONS

Climate warming changed the phenology of many tree species. In case of silver birch, our investigations in North-East of Romania revealed that sap exudation occurs at the same period every year, independent of weather conditions. Almost 65% from total quantity of sap was harvested between 25th of March and 5th of April, as in other European countries, including some northern and central ones. Sap yield was significantly correlated with tree height but not with dbh or age, parameters that usually sap collectors take as indicators of a higher harvest.

The tapping of silver birch trees in consecutive years, induced a significant loss of vigor that reduced productivity with 20%. Tapping trees must be done without compromising their growth and health over time. Therefore, it is essential to exploit the trees in the forest with responsibility, avoiding sap harvest in consecutive years and limiting the collection to one week.

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EFFECT OF PLANTING DATE ON ANEMONE PLANTS, GROWN IN A SUSTAINABLE GARDEN IN BUCHAREST, ROMANIA

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Abstract

Corms of a mixed cultivars of Anemone coronaria ('Mr. Fokker', 'Sylphide', 'Hollandia', 'The Bride', 'Harmony White') were planted in raised beds during the fall, winter and spring. Without applying any maintenance except weed control, plants from the corms planted in November had the most flowers and managed to survive best during the two years of observations. Anemones planted in February and March were able to flower in June and July, when the air temperature was over 30°C. In conclusion, Anemone coronaria proved to be an excellent species for spring conditions in Bucharest, with an extended flowering period, much more than other species such as tulips, daffodils or hyacinths. Moreover, regardless of the planting date, anemone plants are suitable for sustainable flower plantations.

Key words*: Anemone coronaria; climate changes; flowerbeds; phenology events.*

INTRODUCTION

Flowers are important color components of urban green spaces. Compositions with flowers contribute to the aesthetic quality of streets (Todorova et al., 2004) and significantly attract urban population to visit parks (Mou et al., 2023). Moreover, flowerbeds proved to have important effects on humans' aesthetic experience, being able to link them to particular location (Poje et al, 2013). Also, in urban areas, flowers have a positive effect on both diversity and richness of bees (Tommasi et al., 2004; Gunnarsson & Federsel, 2014; Burr et al., 2016; Lanner et al., 2020; Ayers & Rehan, 2021) and butterflies (Nagase et al., 2011).

Studies conducted in different countries revealed that compositions with higher flower color diversity are more appreciated by people (Todorova et al., 2004; Hoyle et al., 2018; Tomitaka et al., 2021). These can be created by using a mix of flower species and/or cultivars. However, in mixed flowerbeds, the combination of species and cultivars became a changeling issue, in the context of climatic changes. Flower species respond differently to climate change (Miller-Rushing & Primack, 2008; da Silva et al., 2014; Sanczuk et al., 2022). Many of them, have changed their phenology events, especially in temperate climate (Tooke & Battey, 2010) and for mixed flowerbeds it is important to select species with a similar response for maximum design effect.

Early spring-flowering species are more responsive to climate change (Fitter et al., 1995; Fitter & Fitter, 2002; Dunne et al., 2003) and this has an important impact on their cultivation in green spaces.

For spring-flowering geophytes, planting date is essential for plant survival, flowering and dormancy, especially in temperate climate. In Romania, where long, warm and dry autumns are followed by short, but cold winters, the planting time of spring-flowering geophytes is quite difficult to establish.

In the actual context, characterized by climate change and energy crisis, it is crucial for urban green spaces to select species and cultivars with adaptive potential and to cultivate them in a sustainable plantations.

Anemone species are naturally adapted to survive summer drought and high temperatures (Kamenetski, 2004), but in Romania are less popular than tulips, daffodils or hyacinths. Flowers of *Anemone coronaria* L. exhibits various colours including purple-blue, red, white and pink and at least 30 gradual colour variants (Dafni et al., 2020). These characteristics recommend them to sustainable urban gardens. But even if the species or cultivars are very adaptative, flowering duration depends on planting date. For this reason, in this study, different planting date were tested on a mix of cultivars of *Anemone coronaria* L. Their performance was investigated in a sustainable urban garden in order to evaluate emergence, growth, flowering, dormancy, plants resilience and response in the second year of cultivation.

MATERIALS AND METHODS

Experiences were conducted in a sustainable urban garden at the University of Agronomic Sciences and Veterinary Medicine, in Bucharest (latitude 44°24'49"N and longitude 26°05'48"E), Romania. Anemone tubers (*Anemone coronaria* L. De Caen Group) from different cultivars ('Mr. Fokker', 'Sylphide', 'Hollandia', 'The Bride', 'Harmony White') were planted at four different times, on November 2020, December 2020, February 2021 and March 2021. Until planting, the tubers were stored at 18-22°C.

Prior planting, the existing soil was improved with leaves compost for a lighter texture.

Tubers were planted without any prior treatment, at a depth of 6 cm, and distances of 20 cm apart within rows and 10 cm apart between rows, in raised flowerbeds, in full sun.

Even if winters in Romania have become shorter in last decades due to climatic changes, they can be very cold or thermally unstable, with dramatic alternations of warm days and very cold ones, which can destroy the tubers. For that reason, after planting, in November 2020 and December 2020, tubers were covered with a layer of 10 cm of leaves mulch, which was removed on 15^{th} of February 2022, when the temperatures rose.

The plants were cultivated without applying any maintenance, except weed control. Observations and measurements were made daily. After the first growing season, the tubers were left in place for the second year of observations.

Statistical analysis were applied to evaluate the plants parameters. The influence of planting date on plants emergence, growth, flowering and dormancy was analysed using one-way ANOVA test, then statistical differences between means were estimated with the Least Significant Difference (LSD) test at 5% level of significance. Also, in order to establish the relationship between flowering parameters (flower bud initiation, number of flowers, flower diameter, stalk length) and air temperatures, linear regression and Pearson test were performed.

RESULTS AND DISCUSSIONS

Planting date influenced tubers emergence in spring (Table 1). For tubers planted in November and December, non-significant ($P>0.05$) differences in the number of days (on average 6 days) from planting to emergence were noticed. Conversely, planted in February, tubers emerged after significantly more days (on average 20 days) than those in March.

Anemone tubers planted in late fall and early winter (November and December) emerged in spring at 2.5 weeks apart, and those planted in late winter and early spring (February and March), started to emerge in the same time (Table 2).

As other early spring geophytes, *Anemone coronaria* start to grow under particular ecological conditions. Emergence and growth of anemones is controlled by temperatures (Ohkawa, 1986), particularly by soil temperature (Mondoni et al., 2009; Rauter et al., 2022). Therefore, even though the first tubers planted in November emerged in February, most of them (60%) emerged in March, when the soil temperature rose.

The mix of anemone cultivars had a more uniform emergence when planted in the spring, compared with those planted in the fall or winter, where some tubers appeared two months apart from the firsts.

However, the share of emerged tubers was best when planted in November and March, of 82.5% and 80.0%, respectively. Early winter planting (December) and late winter planting (February), when the temperatures frequently varied from abnormally high to extremely cold and freezing, resulted in a lower survival of tubers.

In geophytes, relatively high air temperatures lead to faster growth (Khodorova & Boitel-Conti, 2013), but in our study at temperatures of 18°C, tubers planted in March, initiated first flowers after 36.96 days, significant much more than those planted in February (Table 1).

Table 1. Phenological response of anemone plants at different planting date

Planting month	Mean number of days from planting to emergence	Mean number of days from emergence to bud initiation	Mean number of days from bud initiation to flowering	Mean number of days from open flower to wilt flower	Mean number of days from last flower to dormancy	Mean number of days from emergence to dormancy
November	123.78 a	42.86 a	7.80 a	7.60 a	41.91 a	104.72 a
December	117.27 a	35.78 a	8.02 a	6.15 b	28.91 b	90.77 b
February	65.38 b	27.18 b	8.07 a	5.57 c	29.21 b	77.29 c
March	44.21 c	36.96 a	7.22 a	5.03 c	25.45 b	79.38 c
LSD	*9.76*	*7.85*	*1.08*	*0.93*	*10.93*	*11.14*

LSD - Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

Table 2. Emergence parameters of anemone corms, planted in different months

Planting month	Emergence period (date)	Emergence duration (days)	Share of emerged corms (%)
November	22 Feb. - 13 Apr.	51	82.5
December	10 Mar. - 22 Apr.	44	55.0
February	16 Apr. - 17 May	32	65.0
March	16 Apr. - 19 May	34	80.0

Anemone plants had approximately the same mean number of days from bud initiation to flowering, independent on planting date. Anyway, there were differences among cultivars, some of them ('Sylphide', 'Hollandia') required only 4-5 days from flower initiation to flowering and some others ('Mr. Fokker', 'The Bride'), 10-12 days. These results confirm other studies, which revealed that geophytes cultivars perform differently at flowering (Ahmed & Khurshid, 2004; Addai, 2011; Bock et al., 2015; Mohsin et al., 2018), due to their genetic background.

The flowering was longer for anemones planted in March, starting from 26th of May to 7th of July, covering a period of 84 days (Figure 1, Table 3). Shorter flowering periods were recorded at those planted in November (56 days) and December (60 days).

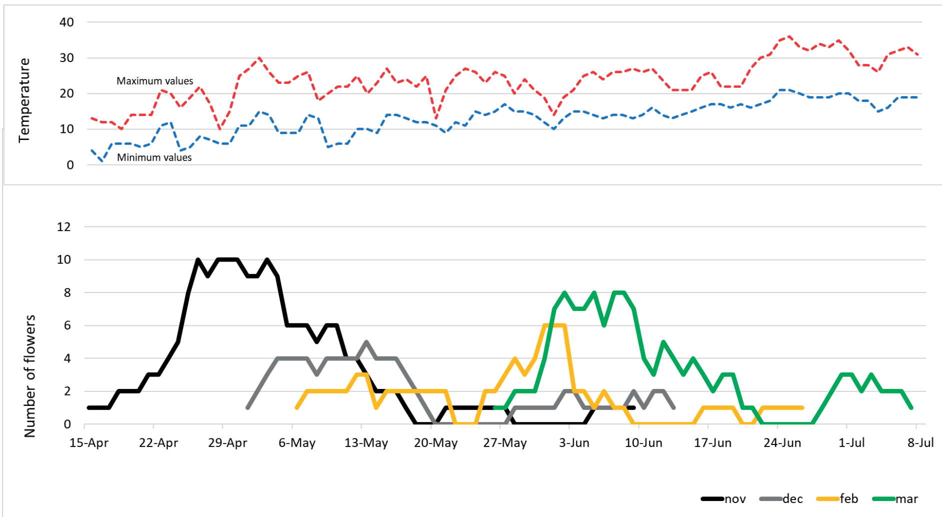

Figure 1. Flowering dynamics of anemones planted at different months

Table 3. Effects of planting date on flowering and flowers parameters

Planting month	Flowering duration (days)	Number of flowers/ plant	Stalk length (cm)	Flower diameter (cm)
November	56	3.26 a	21.56 a	8.11 a
December	60	1.95 b	21.22 a	7.69 a
February	73	1.65 b	19.93 a	6.81 b
March	84	3.13 a	22.72 a	7.50 a
LSD	-	*0.96*	*2.94*	*0.64*

LSD - Least Significant Difference. All data within columns with the same letter are not statistically different at $P \leq 0.05$.

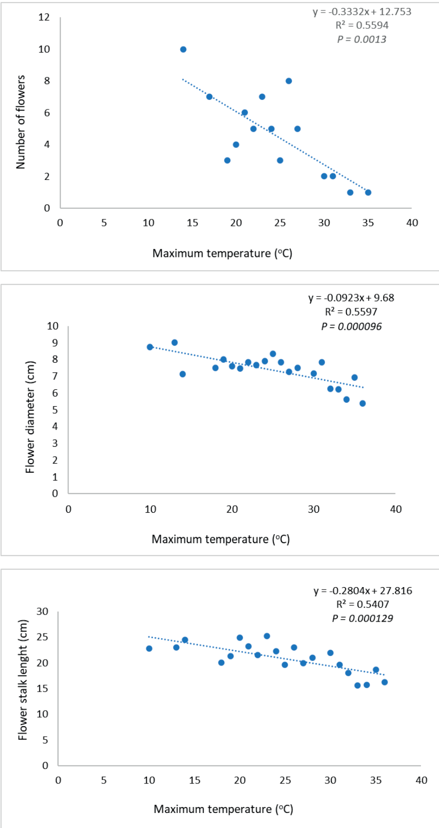

Figure 2. Relationship between flowering parameters and maximum temperature

Comparing these data with those obtained in Romania for other early-flowering geophytes (Manda & Nicu, 2011), it was noted that anemones have a significantly longer flowering (68.2 days) than *Hyacinthus* (10.2 days), *Narcissus* (12.5 days), *Tulipa* (10.4 days), *Crocus* (17.0 days), *Allium* (25.7 days) or *Muscari* (30.2 days).

The length of the flowering period did not influence the number of flowers/plant. Thus, anemones planted in March had almost the same average number of flowers/plant as those planted in November (Table 3).

Significantly lower number of flowers/plant were observed in case of anemones planted in December and February. Data indicate that the number of flowers/plant is rather controlled by environmental factors than length of flowering period or cultivar. In geophytes, temperature was found by many researches the most important factor controlling their flowering (Khodorova & Boitel-Conti, 2013).

In fact, significant relationships (Figure 2) were found among maximum temperature and number of flowers (R = 0.7479; R^2 = 0.5594; P = 0.001344), flowers diameter (R = 0.5596; R^2 = 0.5597; P = 0.000096) and flower stalk length (R = - 0.7393; R^2 = 0.5407; P = 0.000129). Anemone cultivars formed more flowers when the daytime temperature was between 14-26°C. Also, temperatures lower than 19°C in April and May, and also, higher than 25°C in June and July, had a significant impact on flowers diameter and stalk length. Thus, at these temperatures, the flowers were smaller in height and diameter. Even if the flowers were initiated in short days of April or long days of June, their size appears to be controlled only by temperature. This confirms the results of other researchers that in anemones, photoperiod does not control flowers quality (Kadman-Zahavi et al., 1984; Ohkawa, 1986; Ben-Hod et al., 1988; Rauter et al., 2022).

Anemone plants continues to initiate flowers even the temperatures exceed 28°C and succeed to bloom at 36°C in July.

Flower lasted significantly longer in anemones planted in November (on average 7.60 days) and December (on average 6.15 days), than those in February or March (Table 1). For some of the cultivars, such as 'Mr. Fokker' and 'Hollandia', flowers wilt after 10-11 days. Anyway, in the summer, even these cultivars had their flowers withered after 6-7 days.

Plants dormancy was observed at different dates for each planting month (Table 4). This was extended with each subsequent planting month. Consequently, March plants entered dormancy last. However, due to high temperatures and long days (Ben-Hod et al., 1988), these plants recorded the shortest period from the last flower to dormancy, of 25.45 days (Table 1).

November plants started their dormancy 41.91 days after the last flower, at a significantly longest period compared to the other plants. Also, they had a significantly longer growing season (on average 104.72 days), than the others (Table 1).

Dormancy length was significantly longer in November plants than the others (Table 4).

Table 4. Effects of planting date on flowering and flowers parameters

Planting month	Starting of dormancy (date)	Dormancy duration (days)	Survival (%)
November	22 June	110.15 a	63.64 a
December	25 June	103.13 b	68.18 a
February	1 July	105.30 b	38.46 b
March	14 July	99.80 b	15.62 c
LSD	-	*6.93*	*4.76*

LSD - Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

After only 99.80 days, March plants exit dormancy and at the beginning of October, they started to emerge.

November and December plants survived better after the hot and dry summer (Table 4). Most of the March plants were lost because their pre-dormancy period was extremely short, insufficient for complete carbohydrate storage. Poor tubers survival for late planting anemones was reported also by other authors (Armitage & Laushman, 1990). In geophytes, dormant buds require energy to promote they growth. Therefore, plants depend on sugars, and its storage is essential to maintain their dormancy and promote sprouting (Sheikh et al., 2022). Furthermore, due to a suboptimal level of sugars, March plants emerged in October were lost until spring, although the winter was warm.

In the second year of growth, the tubers started to emerge almost at the same time, independent of the previous year's planting date (Table 5). Also, plants emerged in a significantly shorter period (P = 0.00679) of 20-24 days, than in the first year, of 32-51 days.

During winter 2021/2022 all plants maintained their vegetative growth and in mid-February they already initiated flower buds (Table 5).

Table 5. Phenological response of anemone plants at different planting date, during the second year

Planting month	Emergence period (date)	Mean number of days from emergence to bud initiation	Mean number of days from bud initiation to flowering	Mean number of days from open flower to wilt flower	Mean number of days from last flower to dormancy
November	11 Oct. – 1 Nov.	163.46 a	8.19 a	8.42 a	56.66 a
December	17 Oct. - 5 Nov.	160.50 a	7.74 a	7.37 a	65.50 a
February	13 Oct. – 5 Nov.	125.00 b	8.25 a	8.00 a	66.16 a
March	14 Oct. – 4 Nov.	-	-	-	-
LSD	-	*39.38*	*2.41*	*1.61*	*16.79*

LSD - Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

At that time, maximum temperatures were between 13-18°C. The weather suddenly changed at the end of February, when late spring frost and episodes of snow persisted until the end of March. As a result, the cold weather caused the loss of flower buds.

The first flowers were observed in April for all planting dates. Thus, the flowering period did not differ significantly as in the first year (Table 6). However, in the second year, the number of flowers/plant was higher for all planting dates (Figure 3). More flowers were also obtained in the second year on other geophytes (Cardone et al., 2019).

Mean values of flower diameter and stalk length were quite similar for all planting dates, but lower than in first year.

No significant differences were noticed for flowers duration (Table 5).

All plants entered dormancy in June, when temperatures exceeded 30°C. Comparing with the first year, the plants had a longer period from the last flower to dormancy, of 56-66 days. This may be explained by the hot and dry weather in May 2022. Year 2022 was the hottest European summer (van Daalen et al., 2022). In this particular year, temperatures above 30ºC were recorded in Bucharest, starting on May 13, quite unusual for early summer.

Table 6. Flowering and flowers parameters during the second year

Planting month	Flowering duration (days)	Number of flowers/ plant	Stalk length (cm)	Flower diameter (cm)
November	50	7.71 a	17.93 a	6.03 a
December	46	6.72 a	18.27 a	6.67 a
February	48	4.14 b	14.80 b	7.15 a
LSD	-	*3.19*	*3.40*	*0.71*

LSD – Least Significant Difference
All data within columns with the same letter are not statistically different at $P \leq 0.05$.

Figure 3. Flowering in the first and second year of some cultivars (a - 'Mr. Fokker' in 2021; b - 'Mr. Fokker' in 2022; c - 'Hollandia' in 2021; d - 'Hollandia' in 2022). More flowers were observed in May, on the same plant in the second year compared to the first

Anemones plants were cultivated without irrigation, in order to test their behaviour in the extreme climate of Bucharest. As a result, in May, the heatwave and lack of precipitation over an extended period, stopped earlier the anemones from flowering. However, in these conditions, the plants continued their vegetative growth and entered in dormancy in June, as in the first year.

CONCLUSIONS

Climate changes creates unpredictable weather conditions every year and the seasons vary widely. Some early spring geophytes are more adaptable than others. *Anemone coronaria* proved an exceptional adaptability, facing temperatures above 30ºC and continuing to flower much longer than other geophytes. Also, anemones can survive for many years if left undisturbed, in sustainable plantations.
Our results showed that planting date should be considered for anemones flowering and plants survival over the years. Tubers planted in late fall (November) had a shorter flowering period but with a high number of flowers, in all cultivars. Also, these plants survived and flowered best in the second year as well. Spring planting (March) increased the flowering period, but not the number of flowers and the plants did not survive in the second year.
Planting date may have implications for flowerbeds design effect and should be considered when anemones are associated with other flower species in sustainable plantations. Also, considering the last European summer weather conditions (the hottest and driest), we recommend autumn planting, which ensure not only an earlier flowering, but also enough time for sugars storage and plants survival.

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INDIGENOUS AND EXOTIC PLANTS IN EARLY MODERN ROMANIAN PUBLIC PARKS. ORNAMENTAL VERSUS UTILITARIAN

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Abstract

By the mid and late 19th century, Romanian municipalities from south and east of the Carpathians began to modernize and beautify the cities according to Western models; particularly, by creating public parks similar to the ones designed in European capital cities such as: Paris, Vienna, London, Berlin, Budapest or Rome. Today, many aspects still remain unclear concerning the planting schemes and the plants used to decorate these early modern green public (as well as private) spaces in Romania, and one such aspect refers to the use of indigenous versus exotic plants, as well as ornamental species versus utilitarian ones. To this end, the following paper will look into Romanian public park history in order to illustrate how planting schemes and compositions were designed and indigenous/exotic and/or ornamental/utilitarian species were used in these new and modern public spaces. The research is based on archival documentation, bibliographical, and in situ research and highlights historic (19th and early 20th century) planting schemes, models for the planting of public spaces, species used and reasons for using them.

***Key words**: garden history, public parks, indigenous, exotic varieties, plant collections.*

INTRODUCTION

The choice of plant *palettes* for public parks, private gardens, and different public areas was never arbitrary. Whether it was used for aesthetic reasons (eg. visual effects), educational purposes (Debié, 1992 and Conway, 1996), served as a specific background for certain activities, or was even used in an assumed role to awaken and maintain nationalistic feelings (Panzini, 2015), vegetation has always been the main component of a park. Different countries and regions throughout Europe and North America had different approaches to exotic and indigenous, respectively ornamental and utilitarian species and their use in garden and park design (see Debié, 1992; Conway, 1996; Cranz, 1989; Hajós, 2007; Taylor, 2006, etc.).

The following chapters will briefly look into European and North American garden histories, particularly into vegetation use for public park design, and afterwards focus on how, during the mid- and late 19th century and the early 20th century, private gardens and especially public parks from Romania (south and east of the Carpathians) were designed and planted, with what particular exotic or indigenous, ornamental or utilitarian species, and why were such species used.

MATERIALS AND METHODS

This paper is based on a research of historic materials found in both public and private archives and libraries from Romania (Bucharest, Iași, Craiova, Pitești, Târgu Jiu, Bacău , Buzău, Brașov etc.) and abroad (British Library - London, UK; École nationale supérieure de paysage de Versailles - Versailles, France; Biblioteca Nazionale Centrale di Roma - Rome, Italy and Technische Universität Berlin - Berlin, Germany); comparisons and overlapping of archival and contemporary plans and photographs; *in situ* visual and comparative research, as well as plant measurements, in several historic public parks and private gardens located in the south and east regions of the Carpathians.

RESULTS AND DISCUSSIONS

The Park Movement - a summary of planting designs in public park history

In the 19th century, in the early stages of the development of the *Park Movement* phenomenon, the vegetation in public parks was chosen primarily for economic and educational reasons. From an educational point of view, the appearance of public parks coincides with the appearance of (public) botanical gardens - planted spaces with a scientific and educational assumed role. The use of flowering plants, flower beds and borders, and exotic species brought from all over the world matched with the idea of educating the lower classes of society, both in botanical gardens and in some specially designed areas of public parks (see Cranz, 1989). Flower beds, borders, and *arboretums* will first appear in Great Britain, both to create a varied décor but especially to educate people about nature and the origins of some species (Debié; 1992; Conway, 1996; Cranz, 1989; Shoemaker, 2001). Later, this model will be adopted in most public parks on the European and North American continents. The art of plant compositions will be perfected towards the end of the 19th century under the direct guidance of Adolphe Alphand and Édouard André in Paris (Shoemaker, 2001).
On the other hand, an attempt was made to preserve, as much as possible, the specimens of trees that already existed on the lands transformed into public parks. Also, most of the planted specimens of trees and shrubs were not exotic species but native species and were procured from the nearest nurseries and/or transplanted from the nearby forests and fields - thereby contributing to the reduction of the costs necessary to create parks. Moreover, the first public parks built in Great Britain or in German-speaking countries were devoid of rich compositions of trees and shrubs (André, 1879). On the one hand, the relatively small number of tall vegetation meant lower costs of design execution and maintenance, and on the other, sunny and warm days being fewer compared to cloudy and cold ones favoured lawns to the detriment of massive tree compositions (Debié; 1992; Cranz, 1989). In other countries located in the centre and especially in the south of the European continent, the climatic conditions will force the authorities to plant more trees than to sod lawns (Debié; 1992; Cranz, 1989; Conway, 1996). In France, however, the reasons behind the planting of quite large massifs of trees and shrubs were due to several factors related to political desire, the vision of specialists, etc. (Mexi, 2023).
At the turn of the 19th and 20th centuries, the vegetation chosen for public parks would begin to be increasingly influenced by the functional zoning of the parks. In this sense, the choice of species will be made from now on especially according to the quantity and quality of the shade it offered, its resistance to stress factors, vigor, life span, and growth rate, and less according to aesthetic considerations (Debié; 1992; Cranz, 1989; Conway, 1996; Mexi, 2023). The species so used will create the framework and context for games and team sports, children's playgrounds, parade and show areas, cycling and carriage areas, etc. (Cranz, 1989; Conway, 1991; Mexi, 2023).
Later, in the first half of the 20th century, the origin of the species used in public parks will often also serve a political role in shaping a nationalist ideology. In this regard, we will list the almost exclusively indigenous plant *palette* used by Bauer in the remodelling project for Schiller Park in Berlin; the flower gardens in the parks of Holland; palm trees (*Phoenix* sp.), umbrella pines (*Pinus pinea*) and holm oaks (*Quercus ilex*) in gardens and townscapes in Italy, plane trees (*Platanus* sp.) in parks and townscapes in most cities in Great Britain; or even the exotic trees that created a Canadian décor in some public parks in Paris (Debié, 1992).
For example, in the United Kingdom, and especially in London, in the 19th century, a real fashion for urban alignments with plane trees developed. The fashion for plane trees will not only be felt in the Kingdom but also across the English Channel, where the French will prefer the geometric trimming of the canopies, thus recalling the geometric shapes of the vegetation in the most famous historic gardens in the Hexagon (André, 1879). The same fashion of plane trees will be taken over in the German-speaking space and, especially, in Vienna, as well as in Italy, but to a lesser extent (Cassetti and Fagiolo, 2003). In the peninsula, the main species to be used will be the rock oak (*Quercus frainetto*), the umbrella pine (*Pinus pinea*) and the palm (*Phoenix* sp.). These species will be used to support a nationalist discourse whose solid foundations were historic relations to the

Roman Empire (Panzini, 2015 and André, 1879). In the German-speaking space, the use of a predominantly indigenous plant *palette* was also part of a nationalist discourse, and in France, North American tree species will support the colonialist discourse (Panzini, 2015; Mexi, 2023).

Abroad, specialists who designed public parks made several experiments to discover which the most suitable plants would be used in their settings, having the goal to reduce transport and maintenance costs and to create local identities (Mexi, 2023). From Lisbon to Budapest, but especially in Great Britain and France, numerous lists of plant species adapted to their use in public parks have been made (Rodrigues, 2017; Hajós, 2007; Conway, 1991; André, 1879). Even when such studies were not carried out by the designers of the parks, they often kept the number of species and specimens they had used or were going to use centralized in tables (Tate, 2001).

Public parks and planting schemes in Romania

"Given the origin of the word tulip from the Turkish-Persian "lâle", in an era when this flower entered the political, symbolic, and decorative life of the Ottoman Empire, we believe that the (Romanian) pilgrim boyars will have brought some bulbs for their gardens as well. […] Among the bulbs brought from the Ottoman Empire are veiled hyacinths, «beaten» tulips, little red or purple veiled *Erysimum*, «royal flowers» or carnations, «rujela» (unidentified species), «tiparoju» (unidentified species), curly geraniums, violets, rosemary, and many more. […] We find oleanders and jasmines brought from the East, flowers from the Americas, or from different parts of Europe, but with Latin names. The geraniums, so present, are inventoried with different varieties and are called «pelarioane». When do these plants start to be cultivated, and when do they get the name they are known by today?" (Vintilă-Ghițulescu, 2015, p. 351).

As shown in the previous quote, exotic plants were not alien to private manor or castle gardens. Whether it's exotic flowers, flowering and fragrant shrubs and vines, or even special trees such as *Pterocarya fraxinifolia* (Cantacuzino estate in Florești, Prahova County) or *Chamaecyparis nootkatensis* (Peleș Castle in Sinaia, Prahova County), 17th, 18th, and 19th century private gardens did not lack exotic plant collections. With few exceptions, as can be easily seen from archival photographs or from the accounts of various foreign travellers in the Romanian countries (Stan and Mexi, 2017), these gardens were not laid out by specialists or, in any case, the patrons' taste for plant clusters (Vintilă-Ghițulescu, 2015), overlapped with a coherent structure that could have been imagined by professional gardeners, landscape gardeners, or architects. We are not certain when exactly popular/local plant names such as «pelarioane», «invoalte» tulips etc. or 19th century scientific plant names used in archival documents changed (Nagy, 2013) because some of them were used even in the late 20th century official documents now found in public and private archives (e.g. *Accacia julibrissin* - probably *Albizzia julibrissin*, *Robinia psaudacacia* - *Robinia pseudoacacia*; *Oxyacantha coccinea* - probably *Pyracantha coccinea*; *Glycinea apios sinensis* - probably *Wisteria sinensis*, «Tufan» - probably *Quercus pubescens*; «cinjer» (unidentified species); «moscherean» (unidentified species, possibly *Fraxinus ornus* - mojdrean), «meschiak» (unidentified species, probably *Betula* sp., etc. (see ANIC, DMBAN, SJAN Iași, SJAN Dolj, SJAN Gorj, SJAN Argeș, SJAN Bacău).

However, it is possible to observe an approach of a specialized vocabulary to the local vocabulary in terms of plant species (e.g. «Salkim» - *salcâm* - *Robinia pseudoaccacia*, «hallun» - *alun* - *Corylus avellana*, «karpin» - *carpen* - *Carpinus* sp., «liliah» - *liliac* - *Syringa* sp., «nutsch» - *nuc* - *Juglans* sp., «skorush sylbatirchi» - *scoruș sălbatic* - *Sorbus* sp., «hallina alba» - *cătina alba* - *Hippophae rhamnoides*, «plute ku frunse mare argintiu» - plute cu frunze mari și argintii - probably *Populus alba*), especially in the case of those foreign specialists invited from abroad to design private gardens as well as public parks. For example, in the case of the Kiseleff and Cișmigiu gardens in Bucharest, landscape gardener Carl Friedrich Wilhelm Meyer, in the numerous lists of plants he made in order to plant both public parks, he alternately uses the scientific names of the era (e.g. *Vitis hederacea* or *Hedera quinquefolia* - today *Parthenocisus* *quinquefolia*; *Isulicia adhadole* - unidentified species), French descriptions for plants (e.g. *Plantes grimpantes*, *plantes toujours verts*, etc.) or Romanian or Romanianized names (e.g. «Castani adevărați» - "True chestnuts", probably *Aesculus hippocastanum*, «*Plute ku frunse mare argintă*» - probably *Populus alba/Populus* sp., «*Kallin*» - probably *Viburnum opulus*, «*Lemkinesk*» - probably *Ligustrum vulgare*/*Ligustrum* sp., etc.) (Mexi et al., 2018).

Regardless of the names used to describe the plants found in gardens and parks or those used to order and buy different species and varieties for new planted spaces, there is a growing appetite for the use of exotic vegetation, especially starting with the mid- 19th century. We can see that if by the middle of the 19th century there were only a few species of exotic trees and shrubs that "timidly" made their appearance in private gardens and public parks in Bucharest, Iași, Craiova and other important cities, planted spaces being designed particularly with indigenous utilitarian species of trees and vines (e.g. *Juglans, Prunus* sp.*, Malus* sp.*, Vitis*) and ornamental species of shrubs and flowers (e.g. *Rosa* sp., *Syringa, Narcissus, Rudbeckia* etc.), by the end of the century and in the first decades of the 20th century, the archival plant lists (corroborated with the species still found *in situ* today) show an increasing interest in bringing exotic species, particularly ornamental, into public parks and private gardens alike.

Referring exclusively to trees, among the most common exotic species used in this period of time (roughly mid-19th and early 20th centuries) we find, first of all, plane trees (*Platanus* sp.) and chestnuts (especially *Aesculus hippocastanum*), and then the bog cypresses (*Taxodium distichum*), Japanese acacias (*Sophora japonica*), dogwood (*Celtis occidentalis* and respectively, *Celtis australis*), catalpa (*Catalpa bignonioides* – in the 19th century, *Bignonia catalpa*) etc.

Along with all these exotic species we find many varieties such as: chestnuts with red flowers (*Aesculus* × *carnea*) or with variegated leaves (*Aesculus hippocastanum* f. *variegata*), beeches with red leaves (*Fagus purpurea*), and pendulous ash trees, with golden or bicolor leaves (*Fraxinus pendula*, *Fraxinus aurea*, etc.) etc. Among the rarities we mention tulip trees (*Liriodendron tulipifera* - mentioned on the lists of planting materials for the Kiseleff Public Garden in Bucharest and the Bibescu Park in Craiova), maclura (*Maclura aurantiaca*), ziziphus (*Zyziphus paliurus*, according to the archival documents) and others. Among the plant species that do not define trees, but which were often found in private gardens and public parks, we particularly mention wisteria (*Wisteria chinensis*), viburnum (*Viburnum* sp.), hydrangeas (*Hydrangea hortensis*), etc. (Mexi et al., 2018; ANIC; SJAN Dolj; SJAN Gorj; SJAN Iași).

Regarding chestnuts and especially plane trees, they almost represent a leitmotif both for private gardens and for public parks designed in the mid- and late 19th century and in the first two decades of the 20th century. This fact is not surprising if we take into account that the fashion for planting plane trees started in London in the 19th century, continued to Paris and then spread to all corners of Europe, defining historic and nowadays urban landscapes such as the ones of the capital city of the United Kingdom, in Paris, Rome, Vienna and so on. Although, if we look carefully at the collections of plants in parks and gardens today, it may be difficult to believe that these trees were indispensable in the landscaping of past centuries, the research of archival documents points to the contrary. In Romania, a relevant example in this sense is represented by the Kiseleff Garden, which currently has no plane trees, but where, in 1849, 40 such trees were to be planted (ANIC, DMBAN, and Mexi et al., 2018). This fact can be caused by several situations that have not yet been identified, but which can be represented by the non-acclimatization of the trees (however unlikely), their cutting over time (or in the 1930s, when the Kiseleff Garden was radically transformed after a project by Friedrich Rebhuhn), or even the fact that they may have never been actually brought and planted in this public park (Mexi, 2023; Mexi and Zaharia, 2020).

Thanks to an extensive research carried out at the turn of the 19th and 20th centuries by the academician Simion Florea Marian (Popa Marian, 2008 and 2010), it is possible to identify numerous species known and used in an extensive area that includes the current territory of Romania, the whole of Bessarabia, the north of Bucovina, eastern Hungary and northern Bulgaria. Studying only the trees for the moment, upon a careful analysis of the academician's research it can be observed that, if for a number of exotic species such as laurel, anise, lemons, etc. there were numerous legends and culinary or medical recipes that were produced with the help of some of their components (Popa Marian, 2008 and 2010), other exotic species are mentioned in passing or not mentioned at all. This detail suggests that they were either not known to the public or had been introduced much too recently and not enough time had passed for them to be carefully studied and passed through the filter of the collective imagination, as in the case of the aforementioned species. An additional argument in this equation is also represented by the fact that certain trees from which fruit, leaves, flowers, etc. were procured, even if they were not acclimatized in this geographical area, they were mentioned in legends and/or recipes, or they were known to the public through international or regional commerce (Iacob, 2012; Mexi, 2023). This fact suggests that, regardless of whether they were in the studied territory or not, they were known by the population. As there is no such information about some of the tree species previously mentioned, it can be argued that they either had not been introduced yet or had been acclimatized too recently.

Another interesting discussion refers to the choice of indigenous or exotic plant species, but already acclimatized for a good period of time in the Romanian landscape, which were to decorate public parks, but also urban alignments of trees. First of all, we emphasize the fact that there were several fashions regarding the choice of the predominant species used in urban planning and planting. These were initially influenced by European models, and then by local specificities, as well as by the latest research in the field of horticulture and urban arboriculture. From a chronological point of view, several historical periods that define the cityscape of Bucharest (the best documented city) can be noted in the researched documentation - periods that are generally also valid for other localities and geographical areas of the country (Mexi, 2019 and 2023). Three historical stages, relevant to this paper, will be presented at this point, emphasizing the vegetal (tree) *palette* used specifically in the urban landscape, inside or outside public parks:

Period I (1800-1840/45)

Dominant species: linden (*Tilia* sp. - usually *Tilia tomentosa*), acacia (*Robina pseudoacacia*), ash (*Fraxinus* sp. - generally *Fraxinus excelsior*), elm (*Ulmus* sp.).

Other species: plane trees (*Platanus* sp.), chestnuts (*Aesculus* sp., particularly *Aesculus hippocastanum*), carob (*Gleditsia triacanthos*), different species of maple trees (*Acer* sp.), oaks (*Quercus* sp. - usually *Quercus robur* or *Quercus cerris*), poplar (*Populus* sp. - generally *Populus nigra 'Fastigiata'*) and fruit tree species such as walnuts (*Juglans sp*. - usually *Juglans regia*), apples (*Malus domestica* - various varieties), plums (*Prunus domestica* - various varieties) etc.

Also, we cannot forget the grapevine (*Vitis vinifera*) - an almost ubiquitous species in the landscape of Romanian cities, as the following quote underlines: "Vineyards, moreover, are mentioned in many documents as: a place to walk, an oasis of coolness, a place of shelter in times of rest, a place of refuge during epidemics." (Vintilă-Ghițulescu, 2015, p. 370 and Chiodaru et al., 1980, p. 157).

Period II (1845/50-1900)

Dominant species: linden (*Tilia* sp. - usually *Tilia tomentosa*), ash (*Fraxinus* sp. - generally *Fraxinus excelsior*), elm (*Ulmus* sp.), poplar (*Populus* sp. - generally *Populus nigra 'Italica'*), plane trees (*Platanus* sp.), chestnuts (*Aesculus* sp.). Other species: carob (*Gleditsia triacanthos*), various species of maples (*Acer* sp.), oaks (*Quercus* sp. - usually *Quercus robur* or *Quercus cerris*), bog cypress (*Taxodium distichum*), yew (*Taxus baccata*), thuja (*Thuja* sp.), tulip tree (*Liriodendron tulipifera*), Japanese acacia (*Sophora japonica*), etc. and fruit tree species such as those previously mentioned. See, for example, Figures 1, 2 and 4. The vine remains a leitmotif for many Romanian cities, but towards the end of the century, especially in Bucharest, but also in other parts of the country, it will disappear as a result of *phylloxera* attacks. Also, during this period, a significant increase in urban mulberry (*Morus* sp.) plantations can be observed - a direct consequence of the development of the silkworm industry (ANIC-REAZ).

In this period it is noted that, in addition to various exotic species used to create different ambiences or interesting plant compositions to highlight a certain statue or perspective, the general plant composition will be mostly based on a selection of indigenous plants that will be procured from nurseries near the gardens or will be brought from the forests in the vicinity of the cities. Sometimes, as in the case of the Cișmigiu Garden, this aspect will be emphasized by its creator himself, landscape gardener Carl Meyer (ANIC-REAZ). Other times, this aspect can be observed after consulting and analysing the lists of plants, as is the case for the Kiseleff and Cișmigiu Gardens, Bibescu Park (ANIC, SJAN Dolj and Mexi et al., 2018) and/or by analysing archival images.

Another important observation refers to the increasingly prominent presence of trees such as plane trees, elms and poplars. If we already discussed plane trees earlier, more observations can be made regarding elms, but especially poplars. Regarding the first species, it was found in sufficiently large quantities both in the forests near most cities, but also, as can be seen from the plant lists still found today in various archives and nurseries of the time (Mexi et al., 2018). Regarding poplars, it is interesting how they were, in a first phase, used by landscape gardeners like Carl Meyer to highlight, most likely after the model found at the Volskgarten (Vienna) (Hajós, 2007), different components of his parks. A substitute for the much better known cypress (Hajós, 2007; Taylor, 2006; Attlee, 2006; Shepperd and Jellicoe, 1986), this tree species has been extensively used in to emphasize various points of interest. However, the poplar will start to be used more and more towards the end of the 19th century and in the 20th century, exactly at the time when, in Rome, large alignments of pines (*Pinus pinea*), oaks (*Quercus ilex*), palms (especially the genus *Pheonix*), as well as cypresses (*Chamaecyparis* sp.) - species which emphasized an important ideological and political message (Panzini, 2015) - were planted. We could speculate - in the absence of clear evidence - that the use of poplars as a substitute for cypresses (and at the same time one of the species often found in wetland areas of cities) can be seen as an attempt to emphasize and legitimize the Latinity of the Romanians - Latinity that is part of the broad discourse of national affirmation from that period (Moldovan, 2013).

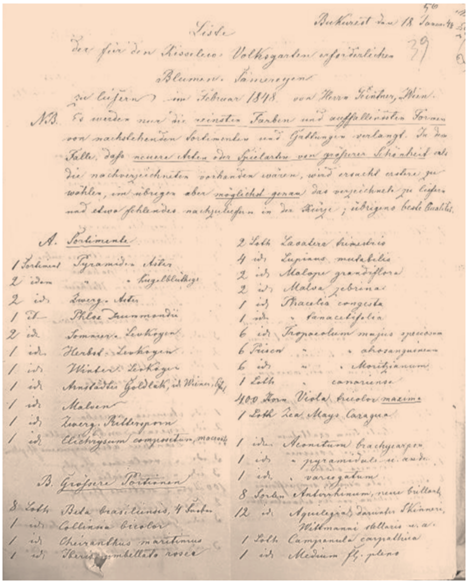
Period III (1900-1930/40)

Dominant species: linden (*Tilia* sp. - usually *Tilia tomentosa*), ash (*Fraxinus* sp. - generally *Fraxinus excelsior*), poplar (*Populus* sp. - generally *Populus nigra 'Italica'*), mulberry (*Morus* sp. - especially *Morus alba*), plane trees (*Platanus acerifolia*), yew (*Taxus baccata*), thuja (*Thuja* sp.), bog cypress (*Taxodium distichum*), dogwood (*Celtis* sp. - generally *Cetis occidentalis*). See, for example, Figures 3, 5 and 6.

Other species: carob (*Gleditsia triacanthos*), various species of maples (*Acer* sp.), oaks (*Quercus* sp. - usually *Quercus robur*, *Quercus cerris*, and more recently *Quercus rubra*), willow (*Salix* sp. – generally *Salix alba*), tulip tree (*Liriodendron tulipifera*), magnolias (*Magnolia* sp.), pines (*Pinus* sp. - usually *Pinus nigra*), catalpa (*Catalpa* sp.), chestnut (*Aesculus hippocastanum*) etc.

During this period we witness an extremely sudden decrease in the use of elms as a result of an epidemic that swept across Europe and devastated all species of elms (*Ulmus* sp.). The famous landscape gardeners Friedrich Rebhuhn also tells about the amplitude of this epidemic in an article from June 1927:

"In all forests and parks, not only in Romania, but throughout Europe, a disease called «the death of elms» struck this species. This disease, which first appeared in the Netherlands, then spread everywhere, slowly killing almost all the elm trees. All attempts to find a means of combating this disease have yielded no results. Three quarters of the old trees in Cișmigiu are elms, all between 60-100 years old, and which are dying partly due to old age, partly due to the disease mentioned above." (ANIC)



Bukurest den 18 Januar 1848

Liste

der für den Kisseleeo Volksgarten erforderlichen

Blumen-Sämereyen

zu liefern im Februar 1848 von Herrn Fischer, Wien.

N.B. Es werden nur die reinsten Farben und auffallendsten Formen von nachstehenden Sortimenten und Gattungen verlangt. In dem Falle, daß neuere Arten oder Spielarten von größerer Schönheit als die nachverzeichneten vorhanden wären, wird ersucht solche zu wählen, im übrigen aber möglichst genau das verzeichnete zu liefern und etwa fehlendes nachzuliefern in der Reihe; übrigens beste Qualität.

A. Sortimente

1 Sortiment Pyramiden Aster
2 idem " kugelblüthige
2 id. Zwerg-Aster
1 id. Phlox Drummondii
2 id. Sommer-Levkoyen
1 id. Herbst-Levkoyen
1 id. Winter-Levkoyen
1 id. Arnstädter Goldlack
1 id. Malven
1 id. Zwerg-Rittersporn
1 id. Elichrysum compositum

2 Loth Lavatera trimestris
4 id. Lupinus mutabilis
2 id. Malope grandiflora
2 id. Malva zebrina
1 id. Phacelia congesta
1 id. " tanacetifolia
6 id. Tropaeolum majus
6 Prisen " "
6 id. " "
1 Loth " canariense
400 Korn Viola tricolor maxima
1 Loth Zea Mays Caragua

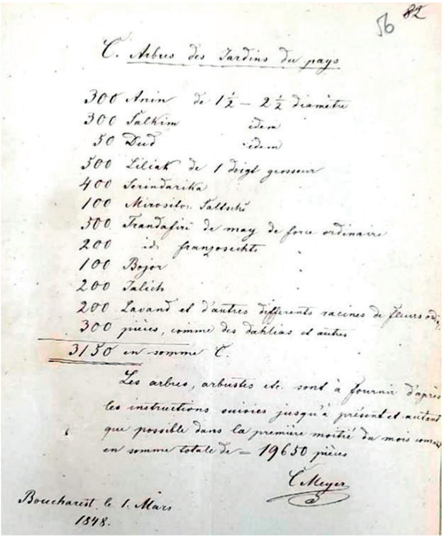
1 id. Aconitum
1 id. " pyramidale
1 id. " variegatum

B. Grössere Portionen

8 Loth Beta brasiliensis
1 id. Collinsia bicolor
1 id. Cheiranthus maritimus
1 id. Iberis umbellata rosea

8 Loth Antirrhinum
12 id. Aquilegia
1 Loth Campanula carpathica
1 id. Medium fl. pleno

Figure 1. List of plants requested for the planting design of Kiseleff Garden in Bucharest, 1848 (ANIC)



56 82

C. Arbres des Jardins du pays

300 Anin de 1½ – 2½ diamètre
300 Salkim idem
50 Dud idem
500 Liliak de 1 doigt grosseur
400 Serindarika
100 Mirositor Saltschi
300 Trandafiri de may de force ordinaire
200 id. franzosechte
100 Bojor
200 Salieh
200 Lavand et d'autres differents racines de fleurs
300 pièces, comme des Dahlias et autres
3150 en somme C.

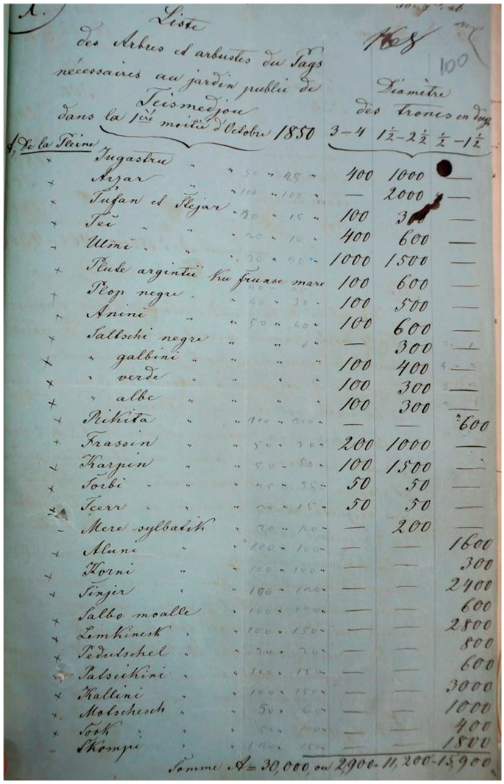
Les arbres, arbustes etc. sont à fournir d'après les instructions suivies jusqu'à présent et autant que possible dans la première moitié du mois courant en somme totale de – 19650 pièces

C. Meyer

Bouchareșt le 1. Mars 1848.

Figure 2. Quantities and special requests for the plants needed for the planting of Kiseleff Garden in Bucharest, 1848 (ANIC)

Figure 3. Planting designs in public parks - Turnu Severin, 1902 (arh. M. Ghigeanu private collection)



Liste

des Arbres et arbustes du Pays nécessaires au jardin public de Teismedjou dans la 1ère moitié d'Octobre 1850

De la Plaine	Diamètre des troncs en doigts 3–4	1½–2½	½–1½
Jugastru			
Arțar	400	1000	
Tufan et Stejar	—	2000	
Tei	100	300	—
Ulmi	400	600	—
Plute argintii	1000	1500	—
Plop negru	100	600	—
Anini	100	500	—
Salcii negre	100	600	—
" galbini	—	300	—
" verde	100	400	—
" albe	100	300	—
Richita	100	300	—
Frassin	—	—	600
Karpen	200	1000	—
Sorbi	100	1500	—
Tesu	50	50	—
Mere sylbatik	50	50	—
Aluni	—	200	—
Korni	—	—	1600
Tingir	—	—	300
Salbo moalle	—	—	2400
Lemn kinesk	—	—	600
Pedulschel	—	—	2800
Salscikiri	—	—	800
Kallini	—	—	600
Molschesch	—	—	3000
Sook	—	—	1000
Skompie	—	—	400
	—	—	1800

Somme A = 30,000 ou 2900 – 11,200 – 15,900

Figure 4. List of trees and shrubs needed for the planting of Cișmigiu Gardens in Bucharest, 1850 (ANIC)

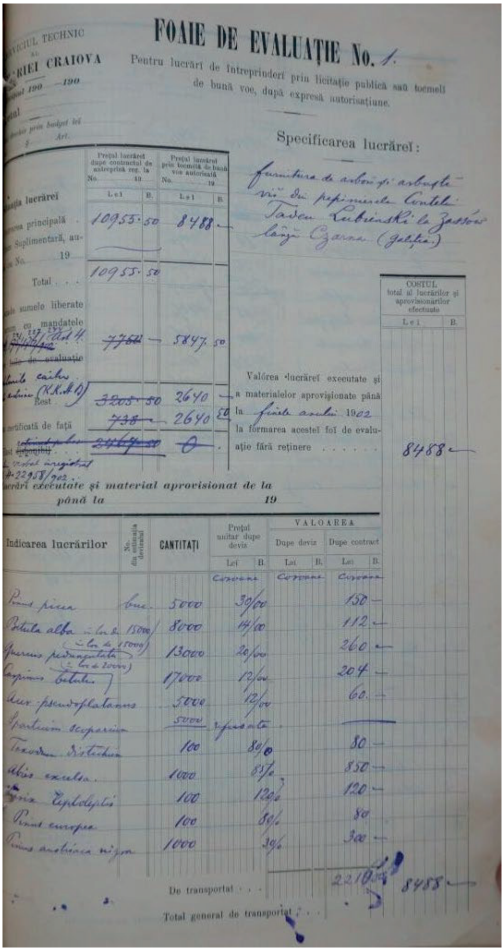

FOAIE DE EVALUAȚIE No. 1.
Pentru lucrări de întreprinderi prin licitație publică sau tocmeli de bună voe, după expresă autorisațiune.
Specificarea lucrărei:
Valórea lucrărei executate și a materialelor aprovisionate până la finele anului 1902 la formarea acestei foi de evaluație fără reținere
Indicarea lucrărilor
CANTITAȚI
VALOAREA

Figure 5. List of plants requested for the planting of Romanescu Park in Craiova from the Count Taven Lubrinsky's nursseries (SJAN Dolj)

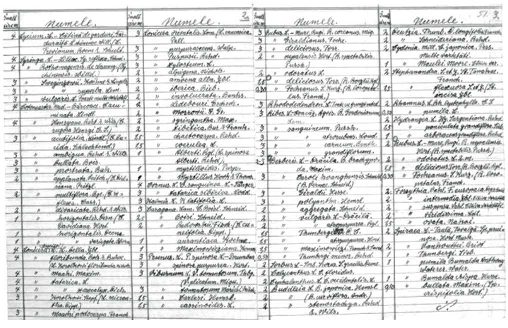


Figure 6. Lists of plants proposed by Fr. Rebhuhn to be planted in public parks and along avenues, undated (ANIC)

CONCLUSIONS

As can be seen, in Romania, the first public parks will represent a continuous horticultural experiment. Unlike countries in the West, where the choice of species depended so much on the (1) physical criteria of the land, (2) on the colour and ambience they created, but also on the (3) message they conveyed, the selection of plants that were to be used in private gardens, but especially in public parks, in Romania will take into account the first two criteria mentioned above and very rarely the third. Often the landscape and horticultural compositions will represent copies of the models already used in Western Europe or North America. Following the Western model, foreign specialists, employyed by the municipality (or third parties) to design parks and gardens, will try to use both native and exotic species (however mostly ornamental and not utilitarian), varieties or cultivars. For example, for the planting of the Cișmigiu and Kiseleff gardens, Carl Friedrich Wilhelm Meyer will create several order lists by which he will request that as many plants as possible be brought to the two Bucharest public parks from the forests in the vicinity of the city and from nurseries inside the city area or nearby, specifying at the same time the quality index that the chosen plants had to meet, as well as the required number of threads/roots/cuttings/bare-rooted or potted plants (ANIC and Mexi et al., 2018). The same thing happened not only in Bucharest, but also in Iași, Craiova, Târgu Jiu, Câmpulung, Buzău etc. (SJAN Gorj, SJAN Dolj, SJAN Iași, SJAN Argeș, SJAN Buzău etc.).

Specialists who came to create public parks in different cities of the country checked the land topography, the quality of the soil, the speed of the water flow etc., before deciding the species of plants based on their ornamental characteristics – as happened, for example, in the case of parks such as the Cișmigiu Garden in Bucharest or the Bibescu Park in Craiova (Redont, 1904; Mexi et al., 2018; Mexi and Culescu, 2018). However, the quality of the planting material, the workers (usually unqualified), poor maintenance, successive redesign interventions, etc. contributed to the fact that only a part of the plant components from the original designs still exist today. This makes those surviving species - almost exclusively represented by trees - to require special attention, especially because certain tree species are no longer produced in nurseries (Mexi and Culescu, 2018).

But referring back to the horticultural experiment, many species of those brought by Meyer, Redont or others will not survive in the city. Their disappearance was caused either by pedo-climatic conditions, different from those of the main (specific) environment (different from the original climate) from which certain exotic species were brought, by local diseases and pests or by those that affected several regions on the European continent, or even by poor local maintenance (ANIC; Mexi and Zaharia, 2020). From the information discovered up to this point, the first specialized study that showed which species could be planted in various areas: urban and rural environments in Romania was carried out in 1957 by the landscape gardener Friedrich Rebhuhn (ANIC; Mexi and Zaharia, 2020). It should be noted that this study, carried out almost a century ago, is no longer valid today as some species have disappeared or are not found in nurseries anymore, the environment, weather, legislation, as well as urban and rural images have changed over time and Rebhuhn's research should thus be revised, corrected and supplemented (Mexi and Zaharia, 2020).

Before concluding the discussion regarding the use of exotic and local, ornamental and utilitarian vegetation, it must be noted that planting was usually the last great work in the construction of a (public) park. Precisely for this reason, sometimes, due to the necessity to inaugurate public parks at a certain well-established moment in time, it happens that the plantations, especially those composed of trees and shrubs, were made only with cut plants. Such a relevant example is Bibescu Park, for the inauguration of which the prefects of the neighbouring counties will be requested by the mayor of Craiova to send cut trees to be planted in the park: "so for now (n.n. 1903) we want to replace the future plantation with an improvised one, of the same appearance." (SJAN Dolj).

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SJAN (Serviciul Județean al Arhivelor Naționale) Gorj, Dolj, Iași, Argeș, Buzău, Bacău, Brașov; various fonds and collections.
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IDENTIFICATION OF MAIN PHENOTYPIC TRAITS OF *DIANTHUS* SPP. CORE-COLLECTION VARIETIES OF PGRB BUZĂU BASED ON FLOWER DESCRIPTORS

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Abstract

Carnation (Dianthus caryophyllus L.) is one of the world's main floricultural crops along with chrysanthemum and rose. Dianthus is one of the oldest ornamental plant in the world. Over time, the aim of Dianthus breeding has been to select or hybridize for totally white or double flowers. BRGV Buzău core collection contains more than 52 varieties of this species, organised in 5 groups according to the type of flower and the direction of use: C group (cut flowers), Gs group (garden type, single flower), Gd group (garden type- double flower), Pd group (pot type-double flower), Ps (pot type-single flower). From the 5 groups, 26 varieties belonging to the following species have been selected for the present work Dianthus caryophyllus and Dianthus chinensis with distinct phenotypic expression. The breeding methods used were clonal selection and repeated individual selection. From selected morphotypes, G I.3., G II.7, G III.6, G IV.3, G V.4 were completely different. The aims of breeding these varieties were the specific clove fragrance, the immaculate white colour, the presence of double flowers, the long vase life and the pleasant commercial and ornamental appearance.

Key words: *morphotype, fragrance, vase life, double flower, carnation*

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is an important ornamental plant that is used as a potted plant as well as a cut flower (da Silva, 2020)

Wild *D. caryophyllus* is likely to have originated in the Mediterranean region (southern Spain, Italy, Sardinia, Sicily, and the Ionian Islands), but the long cultivation history makes it difficult to confirm its precise origin (Takashi O., 2018).

Carnation (*Dianthus caryophyllus* L.) is one of the world's main floricultural crops along with chrysanthemum and rose. It is cultivated year-round in the world's temperate regions, especially in cool highlands, such as in parts of Colombia, China, and Kenya, which are the main production areas (Nimura et al. 2006b; Onozaki 2006).

Carnation breeders are always trying to develop new cultivars with novel colors or other floral characteristics such as variation in the number of whorls or petal margin smoothness, variegation, or even novel leaf
shape and color variation, either through classic breeding, mutagenesis, genetic transformation, or even spontaneous somaclonal variation or mutants (Datta and Teixeira da Silva, 2006).

Caryophyllaceae is one of the largest angiosperm families. It comprises approximately 86 genera and almost 2200 species, which are distributed on all continents but concentrated in the Mediterranean and Irano-Turanian region (İlçim, A. et al., 2013).

The name carnation is derived from the latin tern "Carnatio" meaning fleshness. *Caryophyllous* means pink refers to the colour of blooms of the original species Carnations were cultivated over 2000 years ago (Ali A., 2008). The main breeding targets for ornamentals used to be visual qualities such as appearance, flower color, type, size, and plant form (Boxriker et al., 2017).

Nowadays, vase life of cut flowers has become an important quality factor, and short-lived flowers have limited marketability and consumer appeal (Onozaki T., 2018). Dianthus is one of humankind's oldest ornamental plants (Halmagyi and Lambardi, 2006). Although most carnation cultivars have been bred to produce strong coloring, the absence of such coloring is also desirable for some customers. An important aim in flower color breeding is to obtain pure white (acyanic) flowers in addition to the more familiar colored (cyanic) ones (Mato et al., 2000).

Dalmais, discovered in 1935 the continuous flowering carnation, which formed the basis of the American carnation improvement. The discovery of remontant daffodils in Sicily in 1880 and their subsequent processing through breeding and hybridization contributed to the improvement of remontant carnation varieties for cut flowers and pot culture (Neagu M. et al., 1976).

MATERIALS AND METHODS

The genetic material composed of 56 varieties was selected and systematized in 5 groups: C group (cut flowers), Gs group (garden type, single flower), Gd group (garden type- double flower), Pd group (pot type-double flower), Ps (pot type-single flower). From the 5 groups, 26 varieties of *Dianthus caryophyllus* and *Dianthus chinensis* have been selected as follows: GI1, GI3, GI4, GI5 from C group; GII1, GII2, GII3, GII4, GII5, GII6, GII7,GII8 and GII10 from Gs group; GIII1, GIII2, GIII4, GIII5, GIII6, GIII7 from Gd group; GIV1, GIV3, GIV5 from Pd and GV3, GV4, GV5 and GV6 from Ps group.
The breeding methods used were clonal selection and repeated individual selection. As results of free fertilization of the varieties, were selected some elite plants according to the following goals: specific clove fragrance, pure white colour, double flowers, pleasant commercial appearance, crack resistant calyx, remontant varieties. Statistical calculations were performed using SPSS software, Pearson correlation coefficients were determined as well as variance analysis by ANOVA test followed by DUNCAN test with 95% confidence interval and p-value < 0.05%.
The international descriptors UPOV and IPGRI were used for the phenotypic description of plants.
The RHS colour charts (Royal Horticultural Society, Sixth Edition 2019 reprint) were used for flower colour determination.

RESULTS AND DISCUSSIONS

The flowers of the 26 genotypes studied were described according to the international UPOV and IPGRI descriptors, thus determining the qualitative (Table 1) and quantitative (Table 2) characteristics in order to identify and select genotypes that meet the requirements and aims of the breeding and selection process.

Table 1. Quantitative floral characteristics of the studied genotypes

Genotype	Bud shape	Bud:extrusion of styles	Calyx shape	Calyx: intensity of anthocyanin coloration	Corolla shape
GI1	elliptic	absent	cylindrical	absent	flat
GI3	elliptic	absent	cylindrical	absent	convex
GI4	elliptic	absent	cylindrical	absent	Concave
GI5	elliptic	absent	cylindrical	absent	Convex
GII1	elliptic	absent	cylindrical	absent	Concave
GII2	elliptic	absent	cylindrical	absent	flat
GII3	circular	absent	cylindrical	Very weak	concave
GII4	circular	absent	cylindrical	absent	Flat convex
GII5	elliptic	absent	cylindrical	Very weak	concave
GII6	elliptic	absent	cylindrical	absent	flat
GII7	oblong	absent	cylindrical	absent	Flat convex
GII8	elliptic	absent	cylindrical	absent	Flat convex
GII10	elliptic	absent	cylindrical	absent	convex
GIII1	ovat	absent	cylindrical	absent	Flat convex
GIII2	ovat	absent	cylindrical	absent	Convex
GIII4	elliptic	absent	cylindrical	absent	Concav
GIII5	elliptic	absent	cylindrical	absent	Flat convex
GIII6	elliptic	absent	Funnel-shaped	Very weak	Concave
GIII7	elliptic	absent	Funnel-shaped	absent	Flat convex
GIV1	circular	absent	cylindrical	medium	Concave
GIV3	elliptic	absent	cylindrical	Weak	Flat
GIV5	elliptic	absent	Funnel-shaped	weak	Concave
GV3	elliptic	absent	Funnel-shaped	medium	Concave
GV4	elliptic	absent	cylindrical	weak	Flat
GV5	elliptic	absent	cylindrical	weak	convex
GV6	elliptic	absent	cylindrical	weak	Flat convex

In terms of qualitative characteristics, there is a high variability in the shape of the bud and corolla. However, all flowers show the absence of exserted style and only 2 calyx shapes: cylindrical, in percentage of 85% and funnel shaped, 15%. Also for most flowers it was found that there is no anthocyanin staining on the calyx.

Table 2. Qualitative floral characteristics of the studied genotypes

Genotype	Calyx length (cm)	Calyx width (cm)	Calyx: length of lobes (cm)	Flower diameter (cm)	No. Petals (only for double flowers)	Corolla height (cm)	Petal length (cm)	Petal width (cm)	Style length (cm)
GI1	2.6 a	1 a	1 a	4.6 c	28 a	2.9 b	3.1 c	1.8 b	2.5 b
GI3	2.6 a	1.3 b	0.6 b	6.4 a	29 a	3.3 a	5.3 a	3 a	1.8 c
GI4	2.8 b	1.1 a	0.5 c	5.6 b	23 b	3.3 a	4.2 b	2.8 a	4.2 a
GI5	2.6 a	1 a	0.6 b	4.3 c	23 b	2 c	0.3 d	1.6 b	2.5 b
GII1	2.1 a	0.7 b	0.6 a	6.4 a	-	1.1 c	4.3 a	2.8 b	1.4 a
GII2	2.6 a	1 a	0.5 b	3.8 d	-	1.2 c	3.7 b	2.6 b	1.2 b
GII3	2 a	0.6 c	0.5 b	5 b	-	1.1 c	3.8 b	3.4 a	1.3 b
GII4	1.9 b	0.6 c	0.4 c	4.9 c	-	1.7 b	4.4 a	3.3 a	1.6 a
GII5	2 a	0.6 c	0.3 d	4.5 c	-	1.4 c	3.9 a	2.8 b	1.1 b
GII6	2.1 a	0.6 c	0.3 d	4.8 c	-	0.9 d	3.7 b	3.2 a	0.7 c
GII7	2 a	0.7 b	0.3 d	4.1 c	-	1.4 c	3.4 b	2.4 b	0.8 c
GII8	1.6 b	0.4 d	0.3 d	3.1 d	-	1.2 c	3.2 b	2.7 b	1.1 b
GII10	1.9 b	0.7 b	0.4 c	4.6 c	-	2.4 a	3.9 b	3 a	1.5 a
GIII1	2 c	0.8 b	0.5 b	4.9 c	18 a	1.9 b	3.8 b	1.9	1.7 b
GIII2	3 a	0.8 b	0.8 a	4 c	13 b	1.9 b	4.7 a	1.9	3.1 a
GIII4	2.1 c	0.8 b	0.6 b	5.4 b	6	2 b	4.4 a	3.4 a	1.3 c
GIII5	1.7 d	0.8 b	0.5 b	5 b	10 c	2.5 a	4 a	2.6 b	1.5 b
GIII6	2.6 b	2 a	0.5 b	6.1 a	12 b	3.2 a	4.1 a	2.9 b	1 c
GIII7	2.3 b	1.5 a	0.7 a	5.4 b	8 c	2 a	3.5 b	3.2 a	1.5 c
GIV1	1.6 b	0.7 b	0.5 b	2.9 c	13 b	1.2 b	1.9 c	1.5 b	0.8 c
GIV3	1.6 b	0.6 b	0.5 b	2.5 c	17 a	1.3 b	2.7 b	1.4 b	1.2 b
GIV5	2.1 a	1 a	0.6 a	5.3 a	16 a	1.6 a	3.7 a	3 a	1.5 a
GV3	1.9 b	0.8 b	0.4 c	4.2 a	-	1.8 b	3.1 a	3 a	1.4 a
GV4	2.1 a	1.1 a	0.7 b	2.7 c	-	1.5 c	2.6 b	1.7 c	1 b
GV5	2.3 a	1 a	0.9 a	3.4 b	-	2 a	3.8 a	2.6 b	0.8 c
GV6	1.8 b	0.5 c	0.3 c	2.2 c	-	0.9 d	2.5 b	1.3 c	0.4 c

*letters represent Duncan test results with 95% confidence interval and p<0.05%; CV-coefficient of variation

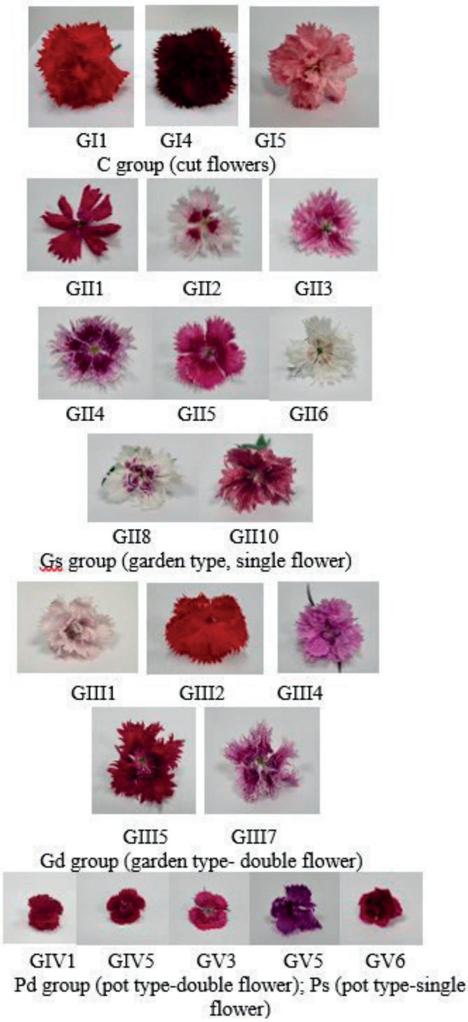

Figure 1. Aspects of floral morphology

In terms of quantitative flower characters, they showed a wide range of variability in the main measurable characters both within each group and between groups that were systematized depending on the direction of use (cut flowers, decorative purpose for gardens), type of culture (pot or garden) and type of flower (single, compound or double) (Figure 1). Following variance analysis of the variants, 5 genotypes were selected that meet the specific requirements and yield certain traits of interest.

GI3 presents flowers that are distinguished by a specific clove fragrance, a suitable habit for cut flowers, a calyx resistant to cracking and long and flexible flower stalks but rigid enough not to fall under the weight of the flower. Flowering is remontant, the flowers are procumbent, allogenous. The flower corolla is concave, the petals have many medium spinose-dentate incisions. Are no stripes or spots on the petals. The ovary is rhombic. The flower colour is RHS 54B indicating deep purplish pink. (Figure 2)

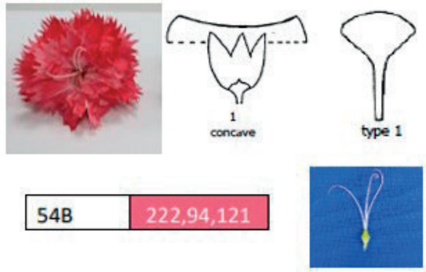

Figure 2. GI3- aspects of floral morphology (flower, corolla, petal, cod RHS, ovary)

GII7 (Figure 3) is a garden type genotype with simple flowers showing pure white flowers, acyanic flowers, flowers that do not accumulate colour pigment. Obtaining pure white flowers was one of the breeding goals at PGRB Buzau because they are a major commercial attraction and can be used in landscaping to brighten up any garden corner. The flower has a flattened convex corolla, medium wavy petals, many deep incisions with crenate dentate edges. The petals have no streaks or spots. The ovary is obovate. Flower colour RHS code NN 155D, yellowish white.

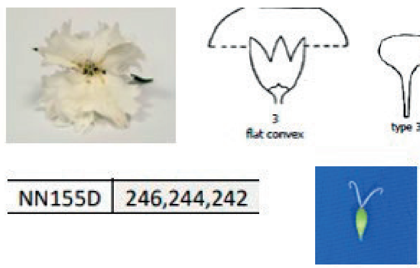

Figure 3. GII7- aspects of floral morphology (flower, corolla, petal, cod RHS, ovary)

GIII6 (Figure 4) is a garden type genotype with compound flowers showing white flowers but with the addition that the petals show a ring of secondary colour. The corolla is flattened, the petals have numerous, very deep incisions and toothed edges. The ovary is ovate. The main colour is NN 155 D, yellowish white and the median ring is 75D, very pale purple.

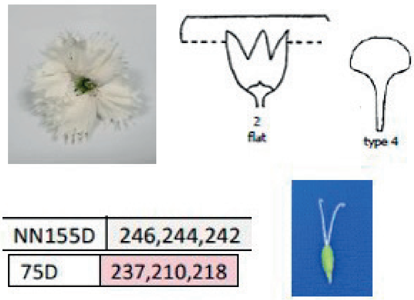

Figure 4. GIII6- aspects of floral morphology (flower, corolla, petal, cod RHS, ovary)

GIV3 (Figure 5) is a genotype of the pot group with compound flowers. The flower corolla is flattened, with medium wavy petals, numerous incisions, toothed crenate type, medium deep. The main colour is 71B, strong purplish red and the secondary colour code 64 A, moderate purplish red, is arranged in widely scattered spots, a large basal spot. The ovary is elliptical.

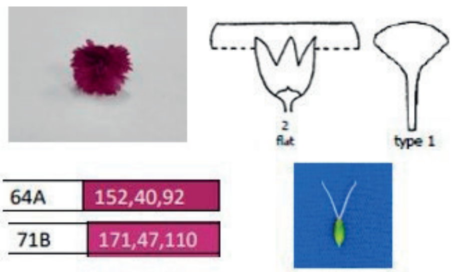

Figure 5. GIV3- aspects of floral morphology (flower, corolla, petal, cod RHS, ovary)

GV4 (Figure 6) is a genotype of the pot type with simple flowers group which shows flowers with flattened corolla, petals with weak and numerous shallow incisions, crenate-dentate and no spots or stripes.

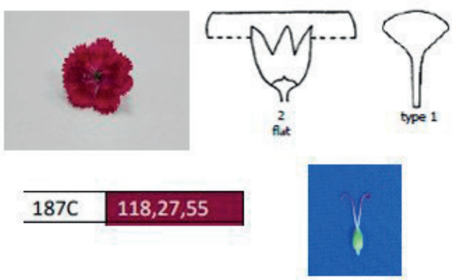

Figure 6. GV4- aspects of floral morphology (flower, corolla, petal, cod RHS, ovary)

The main colour of the flower is 187 C, dark red. The inflorescence is spray type and is the variety dedicated for pot culture as a decorative flower with continuous flowering.
The ovary is oblong.

CONCLUSIONS

The PGRB Buzau germplasm collection belonging to *Dianthus* spp. species has been organized and systematized into 5 groups.
According to the aims of the breeding process, 5 distinct genotypes were selected: GI3, suitable for cut flowers, which has as its strong point the clove scent specific to carnations, G II7, suitable for garden with simple acyanic flowers, GIII6 garden type with compound flowers also from the white flowers group, GIV3, suitable for pot with compound flowers, colour with pleasant commercial aspect and GV4, suitable for pot with simple flowers and pleasant commercial aspect. All genotypes show remontant flowering and protrusive flowers. Breeding will continue with genetic stabilization and selection of material for the purpose of releasing and patenting new varieties.

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PRELIMINARY RESULTS REGARDING THE BEHAVIOR OF SOME *CAMASSIA* SPECIES IN IAŞI ECOLOGICAL CONDITIONS (NORTHEASTERN ROMANIA)

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Abstract

Camassia genus (Asparagaceae family) includes bulbous herbaceous plants native to North America where they have been cultivated for over 7000 years, especially for food purposes. In this paper, the behavior of two species of Camassia (C. quamash and C. leichtlinii), cultivated in the experimental field of University of Life Sciences of Iasi, Romania, was evaluated. The data obtained in the period 2021-2022 indicate the appropriate adaptation of the two Camassia taxa in the ecological conditions of Iași. A greater vigor of the C. leichtlinii plants was evident, respectively taller flower stems, with longer inflorescences, larger and numerous flowers. The correlations between the different morphological characters highlighted the existence of the positive correlation in most cases, stronger being between the height of the stems and the length of the inflorescences, and between the length of the inflorescences and the number of flowers/ stem. Negative correlations in both species were recorded between the number of flower stems/ plant and the number of flowers/ stem. From the phenological point of view, the initiation of vegetation and the appearance of flower stalks took place approximately simultaneously, instead, flowering was about 14 days earlier in C. quamash.

Key words: *camas, ornamental value, characters correlation.*

INTRODUCTION

Geophytes, defined as plants with underground organs specialized in the storage of reserve substances, are known for their importance, especially in food, being valued for their nutritional value, some considering that they could have played a particularly role in man evolution (Fulkerson & Tushingham, 2021; Carney et al., 2021). Also, many geophytes are plants with recognized ornamental value (Cantor, 2016; Toma & Petra, 2020; Draghia, 2011).

This category also includes *Camassia*, which are less cultivated in Romania, although they have special decorative properties. That is why they deserve more attention, as they can represent very good variants in completing the assortment of perennial flowering plants.

Camassia Lindl. is a genus of bulbous plants endemic to North America (Figure 1), identified in 31 US states and three Canadian provinces. The center of diversity for *Camassia*is considered to be southwestern Oregon, given that most species and subspecies of the genus have been identified in this area (Beckwith, 2004). It is an old genus, with shirt pollen being identified in sediments from regions located in the northwestern USA dating back to approx. 70000 years (Thoms, 1989, cited by Beckwith, 2004). Also, in a study of the Late Quaternary specific vegetation of the southwestern Columbia River basin, Barnosky (1985) supports the presence of camasia pollen in two time periods analyzed (33,000-23,500 BC and 8500 BC. - to date).

Camasias are known by various popular names, depending on the species and the area: camasia, Indian hyacinth, wild hyacinth, camash, quamash, etc.

The genus comprises six accepted species (Figure 1), four of which spread across the western North American continent (*C. cusickii* S.Watson, *C. howellii* S. Watson, *C. leichtlinii* (Baker) S. Watson, *C. quamash* (Pursh) Greene) and two in the eastern part (*C. scillioides* (Raf.) Cory, *C. angusta* (Engelm. & Grey) Blank) (Fishbein et al., 2010; Beckwith, 2004; Culley et al., 2013; http://bonap.net/NAPA/TaxonMaps/Genus/County/Camassia;http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=105373; https://wfoplantlist.org/plant-list/taxon/wfo-0000762871-2022-12?page=1)

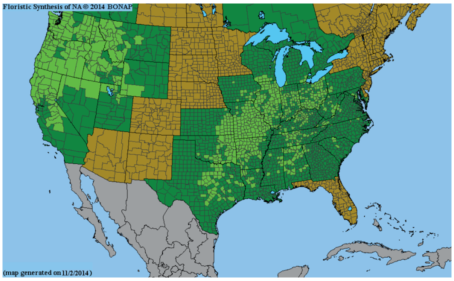


Figure 1. The natural habitats of *Camassia* species (http://bonap.net/NAPA/TaxonMaps/Genus/County/Camassia)
distribution areas areas of origin

Some sources also indicate a seventh species, but unrecognized, *C. engelmanii* Spreng., whose name was first published in 1989, in Bollettino de Societta Toscona d'Orticultura (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:532515-1).

The high morphological variability of the plants, associated with weak genetic differences both between taxa located in the same areas and between those geographically isolated, has justified the cataloging of the genus *Camassia* as a taxonomically difficult group, which has led to numerous studies to clarify species or subspecies delimitation and explain evolutionary mechanisms (Culley et al., 2013; Tomimatsu et al., 2009; Uyeda & Kephart, 2006).

Initially, the genus *Camassia* was placed in the Liliaceaefamily (Gould, 1942; Douglas et al., 2001), then temporarily placed in the family Hyacinthaceae (Dahlgren et al., 1985, cited by Fernandez & Daviña, 1991), but more recent DNA analysis place the genus in the Asparagaceaefamily, subfamily *Agavoideae* (Stevens, 2017; Halpin & Fishbein, 2013; Chase et al., 2009; Archibald et al., 2015; Davis, 2018).

Camasias are geophytes with flowering in late spring - early summer, the underground organ being a tunicate, ovoid or globular bulb, with brown or blackish-brown protective tunics (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=105373). The linear leaves, which appear in early spring, form rosettes, from the middle of which start one or more flower stalks with flowers arranged in racemes. The flowers, actinomorphic or zygomorphic, composed of 6 lanceolate tepals (purple, blue, white), arranged in three, on two vertices, have filiform stamens, with yellow anthers. Fruits are dehiscent capsules with 6–36 black, glossy, ovoid or ellipsoid seeds, 2–4 mm (Douglas et al., 2001; Beckwith, 2004; Proctor, 2013; Davis, 2018; http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=105373).

Camasia bulbs are made up of two components: a mother bulb and a daughter bulb (Thoms, 1989; Beckwith, 2004; Stucki, 2018). The mother bulb acts as a food source for the growing daughter bulb and shrinks throughout the life cycle. The daughter bulb absorbs energy and nutrients from both the mother bulb and the leaves (Maclay, 1928; Thoms, 1989; Beckwith, 2004; Proctor, 2013; Davis, 2018).

After flowering, the above-ground organs disappear and the bulb goes dormant. For spring-flowering, summer-dormant plants, such as camas, the temperature pattern that bulbs require prior to leaf and flower emergence is a warm-cold-warm cycle (Cantor, 2016; Toma & Petra, 2020; Draghia, 2011; Davis & Davis, 2021).

As a rule, to initiate flowering, ornamental geophytes in temperate zones require a short period with temperatures around 20°C (Khodorova & Boitel-Conti, 2013). Prolonged high temperatures in the spring during the above-ground growth period result in earlier flowering, smaller flowers, but also a shorter vegetative growth period, resulting in smaller and less productive bulbs in next year. Low temperatures can prolong above-ground vegetative growth and subsequently bulb growth, but will also result in reduced flowering (Khodorova & Boitel-Conti, 2013; Thoms, 1989; Kuhnlein, 1991; Stevens & Darris, 2001; Russell, 2011). Camasia bulbs show good resistance to low temperatures (up to -30°C), so they wintering in the field. *C. quamash* and *C. leichtlinii* require a period of 80-81 days of cold (approx. 4°C) for leaf emergence (De Hertogh &Le Nard, 1993; Khodorova & Boitel-Conti, 2013).

Based on studies carried out in *C. leichtlinii*, which examined the effect of heat treatment duration on bulb growth and development, it was hypothesized that the daughter bulb and roots develop in the summer, to the detriment of the mother bulb, and that there would be a heat treatment duration after which daughter bulbs stop developing. It has also been estimated that there would be a cooling durationthat minimizes the duration of winter dormancy (Stucki, 2018; Davis & Davis, 2021).

In the spring, until the leaves enter senescence and the seeds have matured, camas have higher moisture requirements, after which, during the summer months, requirements are reduced (Thoms, 1989; Beckwith, 2004; Luna et al., 2008; Kuhnlein, 1991; Stevens & Darris, 2006).

According to archaeological evidence from the Willamette Valley (Oregon, USA), where ancient ovens and remains of charred bulbs have been discovered, it appears that the use of *Camassia* bulbs in food dates back more than 7,750 years, (Aikens, 1993, cited by Sultany & Kephart, 2007).

The importance of camas resides mainly in their food value. Two species of camasia (*C. quamash* and *C. leichtlinii*) were important food sources for the Straits Salish and other Coast Salish indigenous peoples of the Pacific Northwest coast, living in the Canadian province of British Columbia and in the US states of Washington and Oregon (Beckwith, 2004). Bulbs were also a major trade item for the indigenous peoples of this area, often being made intertribally exchanges with camas bulbs, and in western Washington no food except dried salmon varieties was widely traded (Gunther, 1973, cited by Davis, 2018).

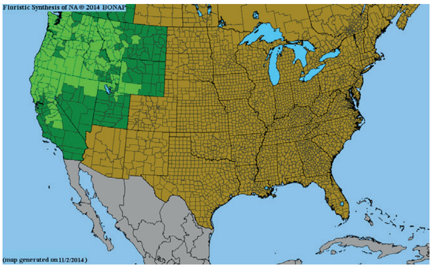
Nutritional analyzes show that camas bulbs have neglctable fat and protein content, but are good sources of fiber, carbohydrates, vitamins, calcium, magnesium, zinc and inulin (a polysaccharide from the fructan class, indigestible and with a less pleasant taste). Apart from its special nutritional and medicinal properties, inulin is believed to provide protection to plants against frost and drought (Turner & Kuhnlein, 1983; Proctor, 2013; Roberfroid, 2005).Camasia bulbs have been consumed for over 8000 years, the most intense period in the production and storage of the bulbs being assumed to be approx. 3000-4000 years ago in the Columbia Plateau area in the states of Washington, Oregon and Idaho (Carney et al., 2021). Archaeological research has established that bulbs were cooked in large ovens using heated rocks, water and vegetation (Proctor, 2013;Stucki et al.,2021; Thoms, 2008a, 2009). Across the southern plains and southeastern forests of North America, the oldest known earth ovens with rock heating elements date back more than 8,000 years. The bulbs of *C. quamash* were harvested in May-June and baked for one or two days in circular hollows on hot stones over which a layer of moistened vegetation was added. Prolonged steaming and the presence of volatile organic compounds from the stratified vegetation ensured the complete hydrolysis of inulin into digestible fructans and fructose (Thoms, 2008b; Proctor, 2013; Roberfroid, 2005; Konlande & Robson 1971; Turner & Kuhnlein, 1983).

From an ornamental point of view, camasia plants can be used in groups or alone, in flower borders or on the periphery of water features, alongside other bulbous or early flowering perennials. It can also be grown in containers to decorate balconies and large terraces, but it must be kept in a frost-free place over the winter. *Camasia* can also be used as a cut flower, lasting about a week.

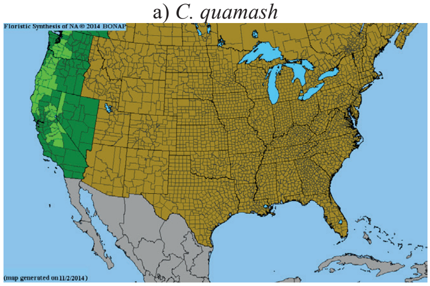
In this paper, the behavior of some *Camassia* taxa cultivated under unprotected conditions, in the north-eastern area of Romania, was evaluated.

MATERIALS AND METHODS

Two *Camassia* taxa were used for this study, respective *C. quamash* and *C. leichtlinii* 'Alba'. Both species are part of the group of camas originating from the western part of North America, with natural habitats in the USA and Canada (Figure 2 a, b).



a) *C. quamash*



b) *C. leichtlinii*

Figure 2. The natural habitats of *C. quamash* and *C. leichtlinii*
(http://bonap.net/NAPA/TaxonMaps/Genus/County/Camassia)

distribution areas areas of origin

***C. quamash* (Pursh) Greene** (Figure 3) is the best-known species, with a wider distribution than the other species of the genus and is characterized by a large morphological variability (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242101517). It has globose bulbs, sometimes clustered, and grey-green, linear leaves, usually fewer than 10 per plant. Flower stems can be 20-80 cm tall, but in most cases do not exceed 50 cm.

Figure 3. *C. quamash* (original)

The flowers, usually zygomorphic, rarely actinomorphic, are up to 4 cm in diameter, arranged in racemes and open sequentially, from bottom to top. They have six blue-violet tepals and the anthers are usually yellow, sometimes blue-violet, purple or brown. After anthesis, the tepals wither separately or remain above the capsules. The capsules are light green to light brown in color and do not fall off after ripening (Douglas et al., 2001; Beckwith, 2004; Stevens & Daris, 2000, http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242101517).

***C. leichtlinii* (Baker) S.Watson**, known as the big camas, has ovoid bulbs, usually solitary, and lanceolate-canalic leaves, about 30 cm long, grouped in rosettes at the base of the flower stalks. The flowers are actinomorphic, with six tepals from bluish purple to bright blue or creamy white, arranged in racemes 30–40 cm long and marked by six filiform stamens, with yellow (sometimes purple) anthers. After anthesis, the tepals twist together and remain over the ovary. Inflorescences can reach 120 cm in height. The capsules are ovoid to ellipsoidal, dull green in color, and when mature, can often do detach from the pedicel (Douglas et al., 2001; Beckwith, 2004; Stevens & Daris, 1999; http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242101516).

Cv.'Alba' (Figure 4) can reach heights of about 100 cm. The flowers have slightly recurved, cream-white colored tepals (Douglas et al., 2001; Beckwith, 2004).

Figure 4. *C. leichtlinii* ‘Alba’ (original)

The establishment of experimental crops was made on 28.10.2021, in the didactic field of the discipline of Floriculture, within the University of Life Sciences in Iași. Bulbs were planted in experimental plots, 40 cm between rows and 35 cm between plants per row.

The experiment was monofactorial, the experimental factor being represented by the species, with two graduations, thus resulting in two experimental variants: V_1 - *C. quamash* and V_2 - *C. leichtlinii* 'Alba'. The organization of the experiments was done in randomized blocks with three repetitions, with 10 plants/repetition. The observations and determinations were carried out from the moment of starting in the vegetation, respectively after 5 months from the establishment of the crops and until the complete drying of the aerial parts. The data focused on the following biometric indices: number of branches per plant, length of flower stalks, number of leaves, length and width of leaves, number of flowers, length and width of tepals. The results were compared with the average of the variants, and the interpretation was made using the analysis of variance, with the "LSD" test (Săulescu & Săulescu, 1967), the control being the average of the experimental variants.

In order to obtain a series of indications on the direction, strength and link between the analyzed morphological characters, an analysis of the correlations between them was also carried out. Grouping the measured data into pairs leads to a first estimate of the common distribution. Scatter plot of values and mathematical modeling by linear regression were used. The general equation for a linear regression model is

$$\bar{Y} = b_1 + b_2X$$

where, b_1, b_2 are called regression parameters. The parameter b_2 represents the expected change in the response $\bar{Y}$ associated with one-unit increase in X. For a given model, the difference between the observed (measured) value of Y and the model-predicted value $\bar{Y}$ at the same given point is called the residual .

This analysis was carried out using the MS EXCEL application from the MS Office 2019 package.

RESULTS AND DISCUSSIONS

The observations and determinations made aimed to identifythe way in which the two camas species develop in the ecological conditions of northeastern Romania and, at the same time, the decorative impact of the plants during the growing season. Since these plants do not only decorate through flowers, but also through beautiful foliage, the study focused both on determining some characteristics of flowers and floral stems (height of flower stalks, length of inflorescences, size of tepals, number of flowers per inflorescence) and number and size of leaves.

Table 1 shows the data recorded on the main morpho-decorative characters of the camasia taxa grown in the experimental field. The results were statistically interpreted and compared with the average of the two variants.

Table 1. The dimensions of some morpho - ornamental characters

Variants	Height of floral stems		Length of inflorescence		Number of flowers/ stem		Diameter of flowers	
	Abs. val. (cm)	Relative val. %/ Signif.	Abs. val. (cm)	Relative val. %/ Signif.	Abs. val.	Relative val. %/ Signif.	Abs. val. (cm)	Relative val. %/ Signif.
V_1 (*C. quamash*)	25.7	70.99 ^{000}	8.9	60.14 ^{000}	13.7	88.39 ^{00}	3.5	70 ^{00}
V_2 (*C. leichtlinii*)	46.7	129.01 ^{XXX}	20.7	139.86 ^{XXX}	17.3	111.61^{XX}	6.4	128 ^{XX}
Average (control)	36.2	100	14.8	100	15.5	100	5.0	100
	$LSD_{5\%}$= 0.2; $LSD_{1\%}$= 0.6; $LSD_{0.1\%}$= 1.8.		$LSD_{5\%}$ = 0.7; $LSD_{11\%}$ = 1.7; $LSD_{0.1\%}$ = 5.3.		$LSD_{5\%}$ = 0.5; $LSD_{1\%}$ = 1.1; $LSD_{0.1\%}$ = 3.6.		$LSD_{5\%}$ = 0.2; $LSD_{1\%}$ = 0.6; $LSD_{0.1\%}$ = 1.8.	

a) b)

Figure 5. The appearance of flower stems (original): a) *C. quamash;* b) *C. leichtlinii* ‘Alba’

Regarding the total height of the flower stalks (Table 1, Figure 5), better results were recorded for *C. leichtlinii* 'Alba' (V_2) which exceeded the average by approx. 29%, the differences compared to the control, being very significantly positive.

The length of the inflorescences (Table 1, Figure 6) was another analyzed indicator, in which V_2 recorded very significantly positive differences, respectively 39.86% above the control values.

Regarding the number of flowers on the stem, the differences between the two variants were smaller, the control being surpassed by the V_2 variant by 11.6%. Apart from the number of flowers/ stem, another aspect must be specified regarding the total number of flowers/plant, was significantly higher in *C. leichtlinii* 'Alba' (47.3), compared to only 13.7 in *C. quamash*, a difference due to a number of 3-5 flowers stems/plant in *C. leichtlinii* 'Alba' and only one in *C. quamash*.

a) b)
Figure 6. Appearance of inflorescences (original):
a) *C. quamash;* b) *C. leichtlinii* 'Alba'

Important differences were also reported regarding the diameter of the flowers (Table 1, Figure 7), the results being in favor of the variant, V_2 with 6.4 cm, compared to 3.5 cm for V_1.

a) b)
Figure 7. Flower detail (original):
a) *C. quamash;* b) *C. leichtlinii* 'Alba'

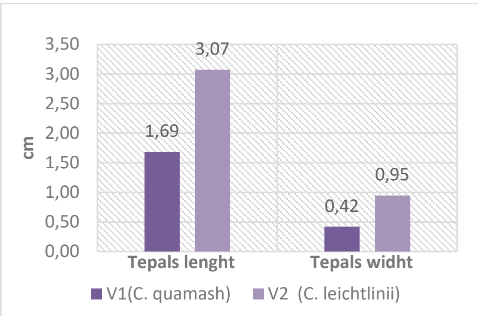


Figure 8. The size of the tepals

From the analysis of the size of the tepals (Figure 8), it can be seen that the average length varied between 1.69 cm at *C. quamash* and 3.07 cm at *C. leichtlinii* 'Alba', and the width between 0.42 cm respectively 0.95 cm.

Leaves complete the decorative appearance of camas plants, approximately 40-45 days before flowering and 30-35 days after flowering (Figure 9).

a) b)
Figure 9. The appearance of the leaves before the floral stems appear (original):
a) *C. quamash;* b) *C. leichtlinii* 'Alba'

The number of leaves/plant ranged from 19.2 at *C. quamash* to 22.3 at *C. leichtlinii*.

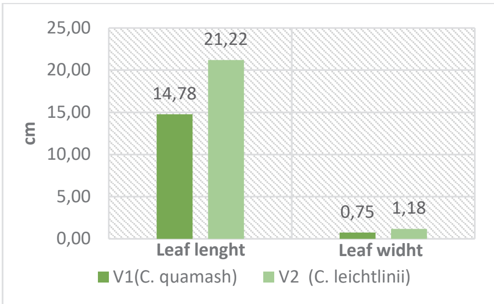


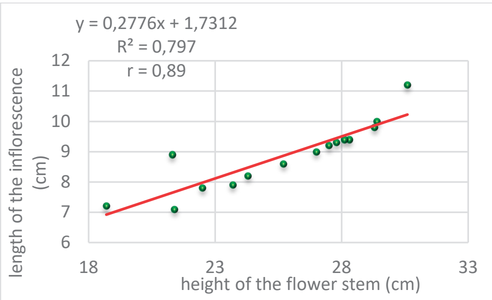
Figure 10. Leaf size

The dimensions of the leaves (Figure 10) varied within wider limits, especially in length, the average values exceeding 20 cm at *C. leichtlinii* 'Alba' (21.22 cm), while at *C. quamash* they were 14.78 cm. Average values of leaf width ranged between 0.75-1.18 cm.
The data obtained as a result of the determinations made were then compared with those from the specialized literature (Table 2) and it was observed that the own results fall within the ranges specified in the bibliography.

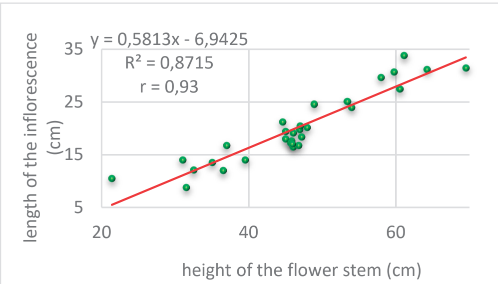
Table 2. The main morphological characters of the studied species (compared to the bibliographic data)

Specification	*C. quamash*		*C. leichtlinii* 'Alba'	
	Personal results (average values)	Bibliography	Personal results (average values)	Bibliography
Height of the floral stems (cm)	25.7	10–70(Beckwith, 2004) 20–70(Douglas et al.,2001) 20–80 (Brickell C., Cathey H.M., 2004)	46.7	20–100 (Douglas et al.,2001; Beckwith, 2004) 60–130 (Brickell C., Cathey H.M., 2004)
Number of flowers per stem	13.7	2–30(Beckwith, 2004)	17.3	4–80(Beckwith, 2004)
Length of tepals (cm)	1.7	1.5–4(Douglas et al.,2001)	3.1	2–4 (Douglas et al.,2001)
Width of tepals (cm)	0.4	0.2–0.8(Douglas et al.,2001)	0.9	0,5–1 (Douglas et al.,2001)
Flowers color	violet		white	
Number of leaves	19.2	Several to numerous (Douglas et al.,2001)	22.3	Several to numerous (Douglas et al.,2001)
Length of leaves (cm)	14.8	20–50 (Brickell C., Cathey H.M., 2004) 15–50(Douglas et al.,2001)	22.8	40–60 (Douglas et al.,2001; Beckwith, 2004) 20–60 (Brickell C., Cathey H.M.,2004)
Width of leaves (cm)	0.8	0.5–3 (Beckwith, 2004) 1–3 (Douglas et al.,2001)	1.3	1–2 (Douglas et al.,2001)

In the conducted study, the existing correlations between different morpho-decorative characters of the two *Camassia* taxa were analyzed and the co-responsible linear regressions were constructed. Pearson correlation coefficients were calculated and regression equations were written.



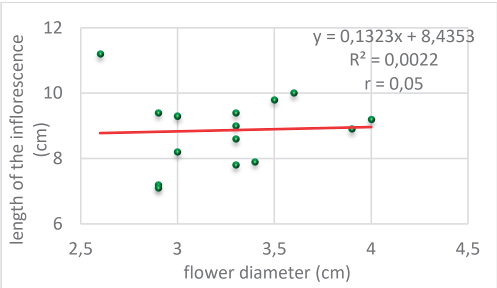
a)



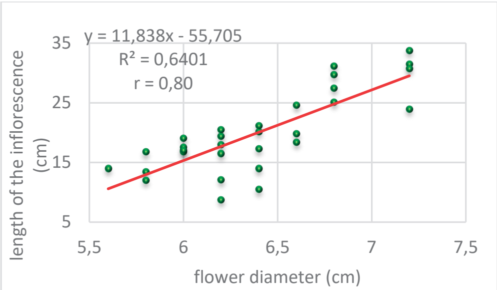
b)

Figure 11. Correlation between floral stem height and inflorescence length: a) *C. quamash;* b) *C. leichtlinii* 'Alba'

Between the height of the floral stems and the length of the inflorescences, very strong positive correlations were identified both at *C. quamash* (r = 0.89) and *C. leichtlinii* 'Alba' (r = 0.93) (Figure 11 a, b).



a)

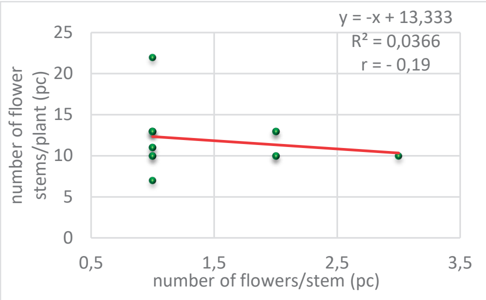


b)

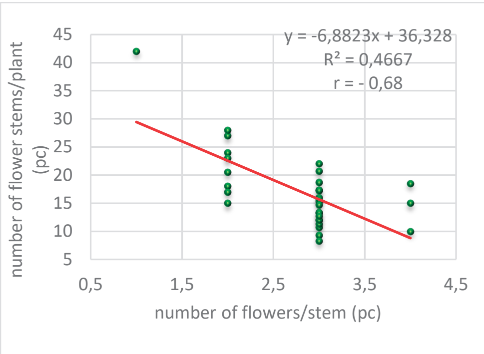
Figure 12. Correlation between flower diameter and inflorescence length:
a) *C. quamash;* b) *C. leichtlinii* 'Alba'

According to the obtained model, the increase of 1 cm in height of the floral stem, causes the increase of the length of the inflorescence by 0.797 cm in the case of *C. quamash*, respectively by 0.8715 cm in the case of *C. leichtlinii* 'Alba'.

Conversely, the correlation between flower diameter and inflorescence length (Figures 12 a, b), although positive in both cases, is non-existent at *C. quamash* (r = 0.05), but strong at *C. leichtlinii* 'Alba' (r = 0.80). Increasing flower diameter by 1 cm leads to 0.6401 longer inflorescence length.



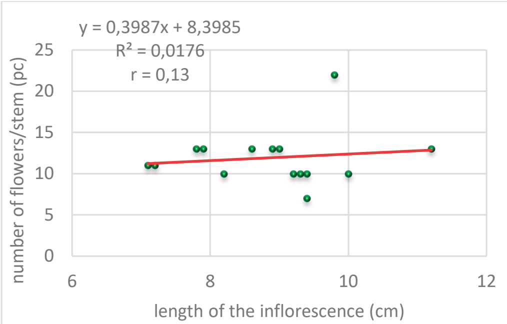
a)



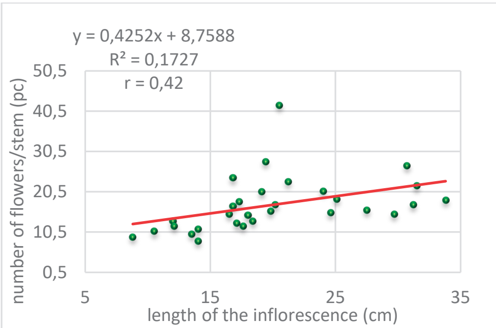
b)

Figure 13. Correlation between the number of flower stems/plant and the number of flowers/stem:
a) *C. quamash;* b) *C. leichtlinii* 'Alba'

For the pair of characters number of floral stems/plant-number of flowers/stem (Figure 13 a, b), the correlations were negative, very weak at *C. quamash* (r = -0.19) and strong at *C. leichtlinii* 'Alba' (r = -0.68). It is observed that when the number of flower stems per plant increases, the number of flowers per stem decreases. Also, according to the obtained model, it follows that in case of the appearance of a new stem, the number of flowers on the stem decreases by 0.4667.



a)



b)

Figure 14. Correlation between inflorescence length and number of flowers/stem: a) *C. quamash;* b) *C. leichtlinii* 'Alba'

Figure 14 (a, b) shows the presence of a positive correlation between the length of the inflorescences and the number of flowers/stem, moderate at *C. leichtlinii* 'Alba' (r = 0.42) and very weak at *C. quamash* (r = 0.13).
The study of the correlations between the different morphological characters highlighted the existence of the positive correlation in most cases, the exception being in the case of the number of floral stems and the number of flowers per stem where, in both cases, a negative correlation was obtained.

For a better highlighting of the development of the main phenophases of the shirts in the first year of culture (2021-2022), the phenological diagram was drawn up (Figure 15). It can be seen that both species start growing in the last week of March. The appearance of flower stalks and flowering register a gap of approx. a week, earlier being *C. quamash*. The flowering period is approximately three weeks, with the two species covering a period of flower decoration between the second week of May and the first week of June.

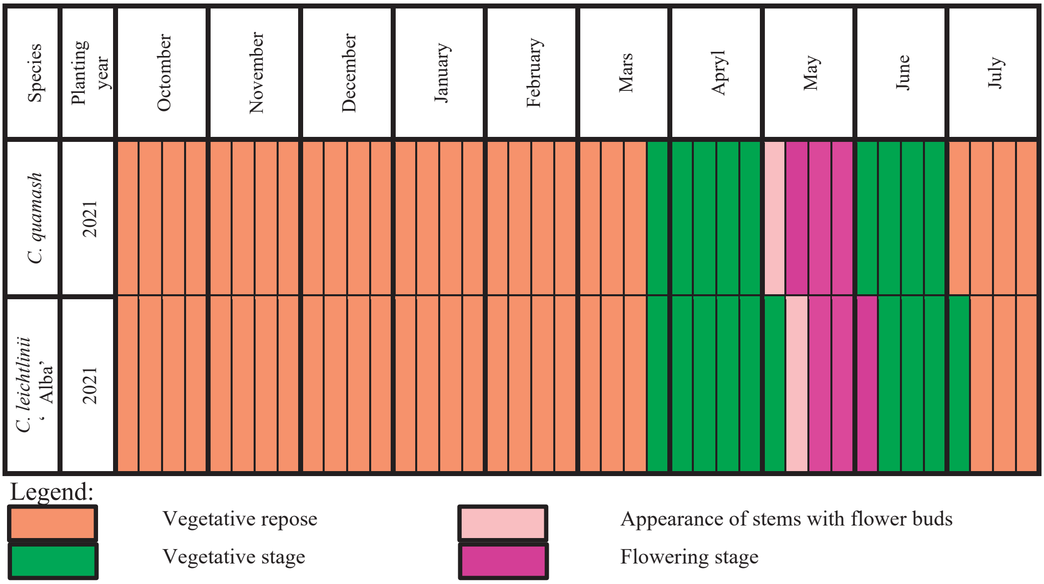


Figure 15. The phenological diagram of camas

CONCLUSIONS

The preliminary data obtained in the period 2021-2022 indicate the appropriate adaptation of the two *Camassia* taxa studied in the ecological conditions of Iași, the results falling within the limits of the specialized literature.
The data regarding the morpho-decorative characteristics analyzed in the camas species indicate a greater vigor of the *C. leichtlinii* plants, expressed by higher values of the number and height of the floral stems (approx. 29% above average), the number and size of the leaves, the number and size of flowers (approx. 11.6%, respectively 28% above average) from the inflorescences.
The study of the correlations between the different morphological characters highlighted the existence of the positive correlation in most cases, stronger being between the height of the stems and the length of the inflorescences and between the length of the inflorescences and the number of flowers on the stem.
87% of the variation in the length of the inflorescence is determined by the variation in the height of the floral stem at *C. leichtlinii*, respectively 79% at *C. quamash*.
Negative correlations in both species were recorded between the number of flower stems per plant and the number of flowers per stem.
46% of the variation in the number of flower stems/plant was determined by the variation in the number of flowers per stem at *C. leichtlinii*.
Flowering takes place from early May to early June (approx. three weeks at each species), the earliest being *C. quamash*.

Depending on the morphological characters, the analyzed species can be recommended, in particular, for ensuring spring - early summer decor in landscaping. At *C. leichtlinii*, the use as cut flowers is not excluded, taking into account the greater length of the flower stems (40-60 cm).

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MORPHOMETRIC ANALYSIS AND ASSESSMENT OF GENETIC DIVERSITY OF WILLOW (*SALIX* SP.) GENOTYPES USING SCoT MOLECULAR MARKERS

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Abstract

In this study, eleven willow genotypes from local populations and foreign varieties grown in an experimental field were morphologically and genetically evaluated to establish their genetic diversity. The results of this study show that the local clonal selections Caracal 101-103 recorded the best results for most of the analysed morphological characters. Out of 25 SCoT primers tested for genetic diversity analysis, 12 primers generated 113 polymorphic bands. The lowest number (8) was obtained with primers SCoT1, SCoT3, SCoT21 and SCoT23. The highest number (13) of polymorphic bands was amplified with the primer SCoT13. The percentage of polymorphism ranged from 72.72% (SCoT 1 and SCoT 21) to 100.0% (SCoT 13) with a mean value of 83.69%. The UPGMA dendrogram grouped willow genotypes into two main clusters. The results of the cluster analysis were then confirmed by the PCoA analysis which explained 61.76 % of the total variation. In conclusion, this study provides valuable data regarding the genetic diversity of willow genotypes that can be selected and used in future breeding programs.

***Key words**: local genotypes, cultivars, morphometry, DNA-markers, genetic diversity*

INTRODUCTION

The genus *Salix* L., is the largest genus in the Salicaceae family, with about 450 species distributed worldwide (Argus, 1997). Furthermore, in Europe *Salix* is considered the largest genus of the woody plant, with approximately 65 described species (Marinček et al., 2023). The high genetic diversity of willow species and their high capacity for vegetative propagation reveal that *Salix* species represent an important crop for woody biomass and renewable fuel production (Horn et al., 2011; Serapiglia et al., 2013; Rönnberg-Wästljung et al., 2022).

In addition to its role as a source of woody biomass, *Salix* sp. plays an important role in the phytoremediation process, contributing to carbon storage, the absorption of heavy metals from the soil, as well as flood mitigation (Weih & Nordh, 2002). Moreover, *Salix* sp. can grow on marginal or less favourable land, thereby reducing land use competition between food and energy crops. The willow trees contribute to the global reduction of natural pollution by presenting a positive CO_2 balance and diversifying the rural and urban landscape (Scordia et al., 2022; Gonzalez- Garcia et al., 2012; Karp et al., 2011). In Romania, as in other European countries, there are many natural hybrids of *Salix* sp., which can be selected and cultivated in Short Rotation Coppice (SRC) crops for biomass production (Botu et al., 2012a; Botu et al., 2010).

In this context, large-scale collection, morphometric evaluation and selection of valuable willow individuals from inter- and intraspecific hybridizations represented important classical breeding activities (Botu et al., 2012b; Botu et al., 2013). Nonetheless, the assessment of genetic diversity based on morphological traits is time-consuming and may be influenced by environmental conditions (Mullis & Falcona, 1987). Conversely, the evaluation of the genetic diversity of willow species by DNA-based molecular markers has been important for the development of non-conventional breeding strategies and their effective implementation for the improvement of willow genotypes in less time (Barker et al., 1999; Daneshvand et al., 2015; Sulima et al., 2017). In this regard, several molecular markers have been used in *Salix* sp. to assess genetic diversity, such as: AFLP (Amplified Fragment Length Polymorphism) (Barker et al., 1999); DArT (Diversity Arrays Technology) (Przyborowski et al., 2013), RAPD (Random Amplification of Polymorphic DNA) (Lin et al., 1994; Sulima et al., 2009; Przyborowski & Sulima, 2010; Sharma et al., 2022), ISSR (Inter-Simple Sequence Repeat) (Van Puyvelde & Triest, 2007; Corneanu et al., 2016; Ghaidaminiharouni et al., 2017, Sharma et al., 2022) and SSR (Simple Sequence Repeat) markers (Barker et al., 2003; Singh et al., 2013).

In the recent past, SCoT markers have been employed in many commercially important and underutilized plant species for a variety of applications, including genetic diversity analysis, interspecific and intergeneric genetic relationships, cultivar identification, mapping analysis, and differential expression of the genes (Rai, 2023).

According to Collard & Mackill (2009), SCoT markers represent a simple, cost-effective, highly polymorphic, and reproducible molecular marker system. Their polymorphism is due to their ability to target the region flanking the start codon, which is considered a highly conserved region in plant genes (Collard & Mackill, 2009).

Thus, the aim of this study was to assess the genetic relationship of eleven willow genotypes using SCoT molecular markers and their morphological characteristics.

MATERIALS AND METHODS

Plant material and measurements of growth parameters

The plant material was represented by the following local clones named Caracal 101, Caracal 102, and Caracal 103 from *Salix* spp. (natural hybrids); Caracal 104 from *S. babylonica* and Caracal 105 from *S. m. tortuosa*, respectively. These clones were selected from an area of the lower course of the Olt River, from the south of Slatina to the confluence with the Danube River.

In addition, four international willow cultivars with complex genetic background such as: Inger (*S triandra* × *S. viminalis*), Terra nova [(*S. viminalis* × *S. triandra*) x *S. miyabeana*], Tora (*S. schwerinii* × *S. viminalis*) and Tordis [(*Salix schwerinii* × *S. viminalis)* × *S. viminalis*] and two other cultivars named Jorr (from *Salix viminalis*) and Lădești 1 (a clonal selection of *Salix* spp. from Romania) were used in this study for genetic diversity evaluation.

A field experiment was organized in Berindei locality (N44°6'44.385; E 24°20'46.714"). The experimental conditions were: brown forest-type soil with pH = 6.0 (0-30 cm) and an average value of recorded precipitation (1100mm/year) in the period 2019 - 2021.The willow individuals in the plots were unirrigated. The field trial was organized in a completed randomized block design (CRBD) with three replications, a single-row system and 15 individuals/ plot. Row spacing was 1.5 m and 1.0 m between individuals/row.

In the second year after planting, the following morphological characteristics were measured in 15 individuals of each genotype: the average number of shoots per plant, the average height of the shoots (cm), and the average diameter of the shoots (mm). To analyse the leaf characteristics of each genotype, 10 leaves/ individual were randomly collected and measured using a portable leaf area meter (LI-3100C, LI-CORGmbH, Germany). The following biometric parameters were recorded: length (cm), width (cm), perimeter (cm), and leaf area (cm^2). All analysed characteristics were subsequently processed as mean values.

Genetic analysis using SCoT markers

The collected leaves from each genotype were dried, ground into a fine powder (TissueLyser II, Qiagen, Germany) and kept at 4°C until the genetic analyses were carried out.

Total genomic DNA was isolated from 0.1 g of dried powder using a protocol based on the CTAB (cetyltrime-thylammonium bromide) method as published by (Lodhi et al., 1994) and improved by (Pop et al., 2003) and (Bodea et al., 2016).

The DNA purity and concentration were determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to performing the PCR (polymerase chain reaction) reactions, all DNA samples were diluted to 50 ng/µL, using sterile double distilled water.

For the SCoT analysis, the PCR amplification reactions were performed using the protocol described by (Collard & Mackill, 2009). The reaction mixture (a total volume of 15 µL) consisted of 50 ng/ µL of gDNA, distilled H_2O for the PCR reactions, 5X GoTaq Flexi Green buffer (Promega, Madison, WA, USA), 1.5 mM $MgCl_2$ (Promega, Madison, WA, USA), 0.2 mM of dNTP mix (Promega, Madison, WA, USA), 1 µM SCoT primer (GeneriBiotech, Hradec Králové, Czechia), and 1U of GoTaq polymerase (Promega, Madison, WA, USA).

The PCR temperature cycling conditions were: (a) 1 cycle of 5 min at 94°C for initial denaturation, (b) 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 2 min, and (c) the final elongation step of 7 min at 72°C.

The PCR amplifications were repeated twice for each SCoT primer to ensure the reproducibility of the results. Separation of the PCR amplified products was carried out by electrophoresis on 1.4% agarose gels (Promega, Madison, WA, USA) stained with RedSafeTM Nucleic Acid staining solution (iNtRON Biotech, Seoul, South Korea) in 1X TBE (Tris Borate-EDTA buffer), at 110 V and 136 mA for 2.5-3 h. The electrophoretic profiles were visualized under UV (ultraviolet) in UVP Biospectrum AC Imaging System (Upland, CA, USA). The list of SCoT primers used in this study is shown in Table 1.

Table 1. The list of SCoT primers used

Primer name	The 3'-5'nucleotide sequence of the primer
SCoT 1	CAACAATGGCTACCACCA
SCoT 2	CAACAATGGCTACCACCC
SCoT 3	CAACAATGGCTACCACCG
SCoT 5	CAACAATGGCTACCACGA
SCoT 6	CAACAATGGCTACCACGC
SCoT 7	CAACAATGGCTACCACGG
SCoT 8	CAACAATGGCTACCACGT
SCoT 9	CAACAATGGCTACCAGCA
SCoT 10	CAACAATGGCTACCAGCC
SCoT 12	ACGACATGGCGACCAACG
SCoT 16	ACCATGGCTACCACCGAC
SCoT 18	ACCATGGCTACCACCGCC

Statistical analysis

One-way ANOVA was performed followed by Tukey's HSD test ($P \leq 0.05$) to determine the statistically significant differences between the means of the analysed morphological traits. Values shown are means ± SE. PAST software (PAle-ontological STatistics Version 4.11, Natural History Museum, Norway) was used to assess the genetic relationships between analysed genotypes based on SCoT molecular markers.

RESULTS AND DISCUSSIONS

Morphological characterization is important in plant breeding, and knowledge of phenotypic trait variations provides valuable information on the genetic variation of traits under improvement (Tharakan et al., 2005).

In this study, the average number of shoots/individual varied between 1.60 (Tordis cv.) and 2.80 in the clonal selection Caracal 101 (Figure 1).

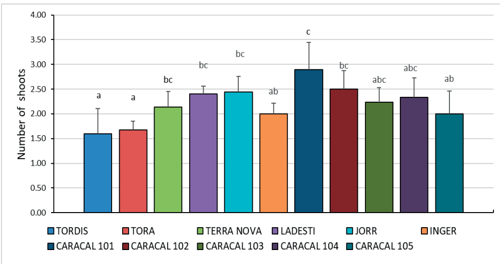

Figure 1. The average number of shoots recorded in eleven willow genotypes

Regarding the average shoot height (Figure 2), it can be seen that the longest shoots were obtained at genotype Caracal 102 (146.41 cm), followed by Caracal 103 (122.89 cm) and Caracal 101 (115.5 cm) with non-statistical differences between their recorded values. The shortest shoots were recorded at Tordis (62 cm) and Lădeşti 1 (58 cm) practically with a height halved compared to the genotypes named Caracal 101, 102, and 103. An explanation of these results is due to the particularities of vegetative development of the analysed genotypes. Tharakan et al. (2005) also reported in a comparative study of 30 *Salix* spp. clones differences in their growth characteristics.

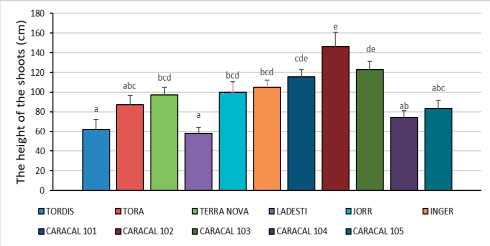

Figure 2. The average shoot height recorded in eleven willow genotypes

Similar to shoot height, the genotypes Caracal 101, 102, and 103 presented the best results in terms of the average of the diameter of shoots (7.46 mm; 8.48 mm, and 7.84 mm, respectively). The lowest mean value of diameter was recorded in Tordis (4.59 mm) and Caracal 104 (4.77 mm) genotypes as shown in Figure 3. It should be noted that Tordis cv. originating from Northern Europe (Sweden) had the weakest morphological development in the environmental conditions of the experimental field organized in Berindei, Romania.

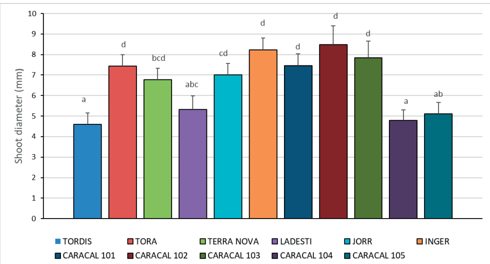

Figure 3. The average shoot diameter recorded in eleven willow genotypes

Leaf morphometry

The longest leaves were recorded in the clonal selection Caracal 102 with 9.94 cm and Inger 10.03 but with statistically non-significant differences. The leaves with the smallest length were measured at Caracal 104 (5.58 cm) followed by Caracal 101 (5.88 cm).

It should be noted that the highest mean value of the leaf width (1.94 cm) was recorded in the Lădeşti 1 genotype, while the lower value was recorded in Caracal 104 (0.77 cm) (Figure 4).

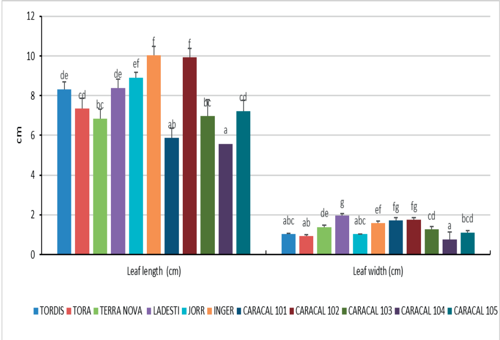

Figure 4. The average leaf length and leaf width (cm) recorded in eleven willow genotypes

The smallest leaf surface (2.83 cm^2) and the smallest leaf perimeter (11.85 cm) were recorded in the Caracal 104 genotype and the best results were recorded in the Caracal 102 both in terms of surface (11.23 cm) as well as the perimeter of the leaf (20.76 cm) (Figure 5).

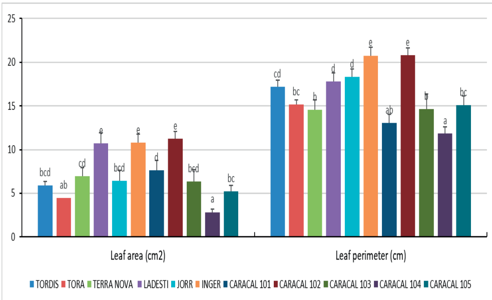

Figure 5. The average leaf area (cm^2) and leaf perimeter (cm) recorded in eleven willow genotypes

After conducting a comparative study of the leaves of the selected clones it was determined that the leaves of the different clones differ in size. In this study, after conducting the comparative analysis of the leaf morphological parameters it can be stated that the leaves of the clones differ in size. Nevertheless, it has been observed that leaf size was also influenced by environmental conditions not only by the biological characteristics of the genotype and its provenance. Our results are in agreement with those reported by Norieka R. (2015) who evaluated the biological characteristics of purple willow (*Salix purpurea* L.) clones and leaf morphometry under experimental short-rotation culture conditions and concluded that environmental conditions influenced the size of willow leaves.

Genetic analysis based on SCoT molecular markers

In Romania, the efforts to improve willow biomass through breeding are currently hampered by the limited information available on genetic diversity and genetic relationships within and between species, clones and hybrids in the gene pool (Corneanu et al., 2016). On the other hand, according to Przyborowski & Sulima (2010) willow hybridisation occurs commonly in nature and the relatedness of many clones is unclear. Thus, molecular biology techniques were applied to analyse the genetic diversity of willow genotypes (Hanley & Carp, 2013).

The results of the present study show that SCoT markers were suitable to assess the genetic relationships between eleven willow genotypes. Out of the 25 primers screened for their ability to amplify the DNA samples from *Salix* spp. genotypes, twelve revealed reproducible and consistent results. The levels of polymorphism detected with selected SCoT primers are presented in Table 2.

Table2. The level of polymorphism detected with SCoT primers in eleven *Salix* spp. genotypes

Primer name	Size of bands (bp)	NPB	NTB	PPB (%)
SCoT 1	450-1750	8	11	72.72
SCoT 2	400-1500	10	12	83.33
SCoT 3	250-1550	8	10	80.00
SCoT 6	350-2300	10	11	90.90
SCoT 12	250-1700	10	12	83.33
SCoT 13	300-2000	13	13	100.00
SCoT 16	250-2000	10	13	76.92
SCoT 18	350-1750	11	12	91.66
SCoT 19	350-2000	10	11	90.90
SCoT 21	300-1500	8	11	72.72
SCoT 22	600-2500	9	11	81.81
SCoT 23	450-1550	8	10	80.00
Total		113	137	
Mean		9.41	11.41	83.69

The twelve SCoT primers amplified 137 reproducible fragments ranging from 250 to 2500 bp, out of which 113 bands were polymorphic bands (9.41/primer). The number of polymorphic bands for each primer ranged from 8 to 13. The highest number of polymorphic bands (13) was generated by SCoT 13 (Figure 6). The lowest number of amplified polymorphic bands (8) was obtained with the primers SCoT 1, SCoT 3, SCoT 21 and SCoT 23.

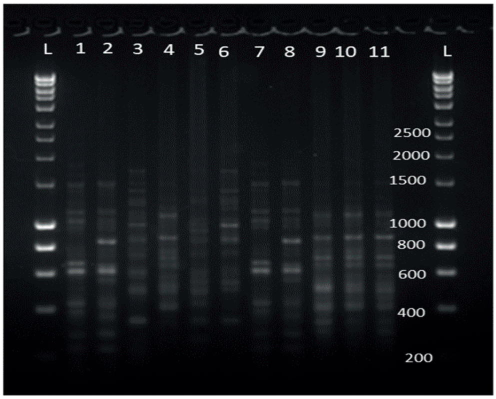

Figure 6. The genetic profiles of 11 genotypes with primer SCoT 13: 1-Caracal 104; 2-Caracal 105; 3-Lădești 1; 4-Jorr; 5-Inger; 6-Terra nova; 7-Tora; 8-Tordis; 9-Caracal 101; 10-Caracal 102; 11-Caracal 103

The percentage of polymorphism (no. of polymorphic bands/ no. of total bands x 100) ranged from 72.72% (SCoT 1 and SCoT 21) to 100.0% (SCoT 13) with a mean value of 83.69%.

In the present study, the genetic diversity between eleven willow genotypes was also assessed by multivariate analysis.

The UPGMA dendrogram, built based on Euclidean distances, grouped the willow genotypes into two main clusters as shown in Figure 7. The first main cluster included two sub-clusters: the first grouped Caracal 105, Caracal 104, Tordis, and Tora; the second sub-cluster grouped the genotypes named Caracal 101-103. In addition, the second main cluster included Jorr, Inger, Terra nova, and Lădeşti 1, respectively. These clustering patterns suggest that local Caracal 104 and 105 clonal selections differ at the molecular level and confirm their morphological differences due to the species of origin.

For Caracal 101-103 genotypes, phenotypic identification was not possible, being probably interspecific hybrids. In a previous study on the assessment of molecular polymorphism in *Salix* spp. accessions from Romania, also Corneanu et al. (2016) identified a high level of

polymorphism both among different accessions of a species and between species.
In this study, the results of the cluster analysis were then confirmed by the Principal Coordinate Analysis (PCoA) which explained 61.76 % of the total variation (Figure 8).

CONCLUSIONS

In this study, the identification of the local clonal selections Caracal 101-103 that presented the best results regarding most of the analyzed morphological parameters represents promising results regarding their use in future willow breeding programs. Cultivation and evaluation of these local clones for several years under experimental field conditions will represent an important objective in promoting these genotypes for biomass production. To our knowledge, this is the first report highlighting the genetic diversity of *Salix* spp. using SCoT markers.

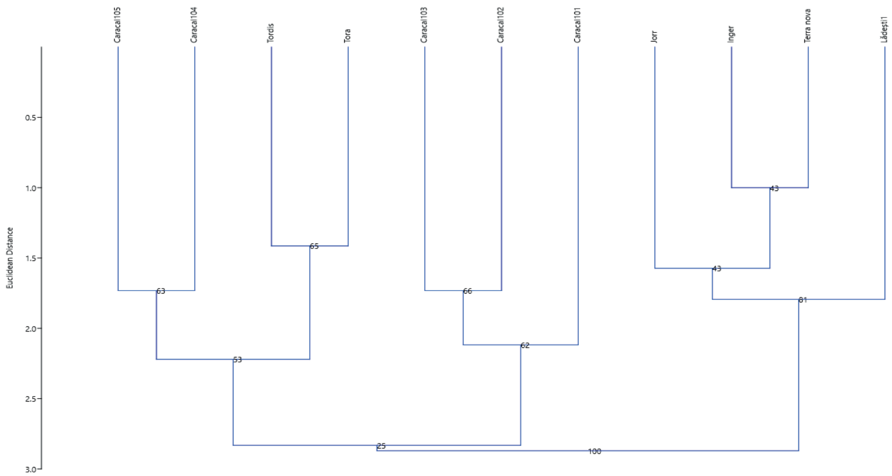

Figure7. UPGMA dendrogram generated by SCoT markers, showing the relationships between eleven willow genotypes and based on Euclidean’s distance index. Numbers on the branches show bootstrap values, computed from 9999 replications

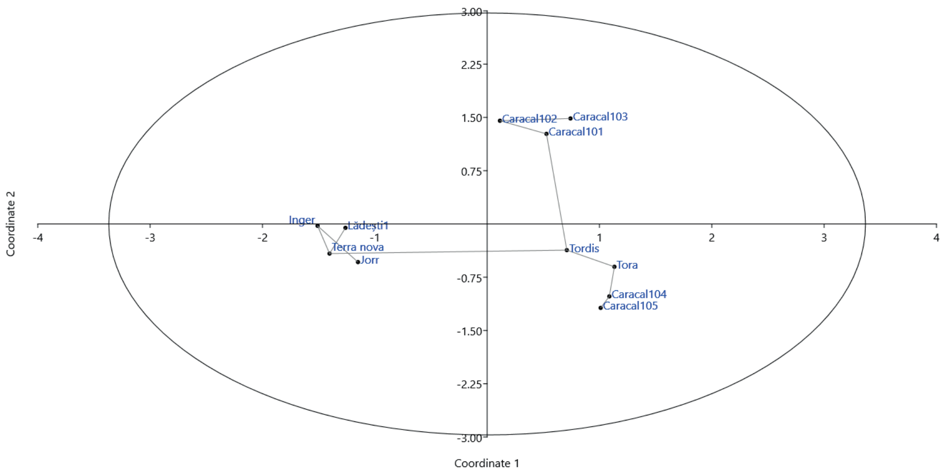

Figure 8. PCoA plot showing the relationships between eleven willow genotypes based on Euclidean distance index

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COMPARING DIFFERENT GRASS SPECIES, VARIETIES AND GRASS MIXTURES IN THE ENVIRONMENTAL CONDITIONS OF TÂRGU MUREȘ

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Abstract

Worldwide the land surface is covered 25–30% by grass. Our objectives were to determine the germination percentage, germination capacity, height, width, softness, disease resistance, density, trampling tolerance, fresh green weight of the different grass species, varieties and grass mixtures under the environmental conditions of Târgu Mures. For the experiment we have selected Festuca arundinacea 'Green dwarf', Festuca arundinacea 'Patron', Festuca arundinacea 'Titanium', Festuca arundinacea 'Greystone', Festuca arundinacea 'Asterix', Festuca rubra commutata 'Rushmore', Festuca rubra rubra 'Livision', Poa pratensis 'Arrowhead', Poa pratensis 'Evora', Poa pratensis 'Baron' varieties. Moreover, four types of lawn mixtures were analysed in the present study: which is suitable for full sunshine and shady places, for regenerative lawn and lawn mixture for sport fields. From the data obtained could be concluded that which selection of grass mixtures suitable for different purposes: ornamental, trampling tolerant. In conclusion, the present work strengthens the possibility of choosing the appropriate grass species, varieties and grass mixtures.

Key words: *grass, lawn mixtures, varieties.*

INTRODUCTION

The lawn is the hearth of the garden (De, 2017) moreover worldwide 25-30% of our land surface is covered with grass. They are considered one of the indispensable parts of gardens, parks, public landscape. De (2017) mentions that it could be named as natural green carpet for outdoor rooms. Lawns and flowers are one of the most important parts of cities (Gladkov et al., 2021) and public areas (Knot et al., 2017; Sehrt et al., 2020). Lawns are human-made habitats in urban green areas (Yang et al., 2019). A common use of the lawn is to increase the stability and erosion protection of embankments, fills and ditches, moreover to be a cover of sports fields and orchards (Nagy, 1978), furthermore they have gained popularity at residential houses, at various companies and establishment buildings, which can add a positive image, a higher standard of living for the owners (Jankowski et al., 2018). Such green urban areas are important parts of human well-being, and their appearance are closely related with the quality of life in cities and the health of their inhabitants (Parker & Simpson, 2018; Tsai et al., 2019). Nowadays lawns occupy 70-75% of the green open spaces of the cities (Ignatieva et al., 2015). According to Tiwari et al. (2015) lawn is an area where individual plants are crowded together and forced into an unnatural growth habitat, however they can absorb atmospheric pollution, remediate contaminated soils, provide cooling by evaporation, and help with the mental health. Even some grass species could be used potentially as phytoextractors of heavy metals, due to fast growth, extensive root system, adaptability to infertile soil (Rabêlo et al., 2018). In a study resulted that *Festuca arundinacea* and *Lolium perenne* are grass species which are least sensitive to polycyclic aromatic hydrocarbons from drilling waste (Gawryluk et al., 2022). Lawns are massively prefabricated elements of the landscape design (Ignatieva et al., 2020). Furthermore, they are mostly used as covering for places after demolition of buildings to increase the aesthetics of the place (Ignatieva & Hedblom, 2018).

Many studies suggest that intensively managed lawns reduce plant and insect diversity in urban areas (Lerman et al., 2018; Rudolph et al., 2017; Watson et al., 2020). Five turfgrass species

showed different germination rate, *Lolium perenne* had germinated in a higher and faster rate compared to the others, and on the other hand the *Poa pratens* recorded the lowest germination (Charif et al., 2019). In a previous study is demonstrated that the mixture grass seeds result in substantial improvement in coverage and density (Robins & Bushman, 2020).
The present paper aim is to determine which grass species, varieties and grass mixtures have good germination percentage, germination capacity, height, width, softness, diseases resistance, density, trampling tolerance, fresh green weight under the environmental conditions of Târgu Mures, to facilitate the selection of grass mixtures suitable for different purposes (ornamental, trampling tolerant, etc.). In this way, knowing the varieties, we can choose the mixtures or varieties that best meet our requirements.

MATERIALS AND METHODS

The experiment was conducted at Sapientia Hungarian University of Transylvania, in Târgu Mureș, between April and November. The soil was a sandy loam and marly clay, which is poorly drained, favouring the formation of stagnant wetland (Table 1). The average temperature during the experiment was 14.7C° (Figure 1) and the average precipitation 53.4 mm (Figure 2).

Table 1. Particle composition of the soil at the experimental site

Sample	1	2	3	4	5
0.25–2.00 mm sand %	19.1	19.3	19.3	17.7	18.6
0.05–0.25 mm sand %	31.4	26.9	20.4	15.6	10.8
Total sand %	50.5	46.2	39.7	33.3	29.4
0.02–0.05 mm silt %	0	0	4.1	10.5	22.9
0.01–0.02 mm silt %	0	0	0	0	0
0.005–0.01 mm silt %	28.8	33	22.9	23	18.8
0.002–0.005 mm silt %	2.1	2.1	0	0	0
Total silt %	30.9	35.1	27	33.5	41.7
0.002 > loam %	18.6	18.7	33.3	33.2	28.9
Depth cm	5–17	20–40	40–55	80–100	110–130
KA	39.1	38.7	41.8	48.8	46.8
Total P mg/kg	2280	1330	1000	860	786
Total K mg/kg	4440	3760	3680	3640	3360

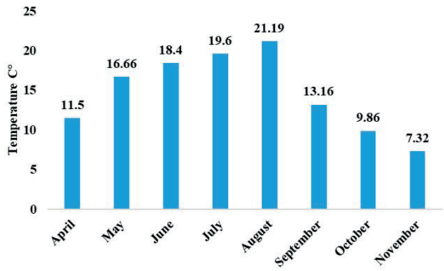

Figure 1. Average temperatures (C°) during the experiment

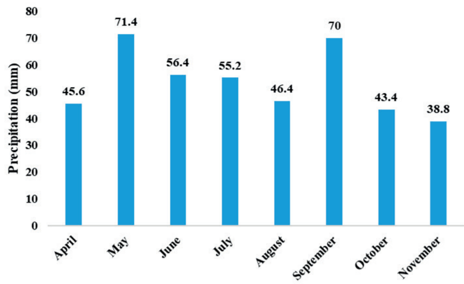

Figure 2. Average precipitation (mm) during the experiment

As plant material there were selected different species, varieties and mixtures of grasses, namely *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB) varieties. Furthermore, four types of lawn mixtures, which were acquired from Agrosel S.R.L.: full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha).
The four types of mixtures percentage distribution of grass mixtures are the following:

- full sunshine lawn (Sun): 40% *Lolium perenne*, 35% *Festuca rubra rubra*, and 25% *Festuca arundinacea*;
- regenerative lawn (Reg): 80% *Lolium perenne* and 20% *Festuca rubra rubra*;
- sports field lawn (Spo): 30% *Lolium perenne*, 20% *Festuca rubra rubra*, 20% *Poa pratensis*, and 30% *Festuca rubra commutate*;
- shady place lawn (Sha): 45% *Lolium perenne*, 20% *Festuca rubra rubra*, 10% *Poa pratensis*, and 25% *Festuca rubra commutate*.

The germination percentage was determined after one week, and the capacity was determined after two weeks after placing in the Petri dish. Germination percentage and capacity were determined by adding the number of seeds germinated and dividing by the number of replicates. Since in each case four replicates were measured, the seeds germinated in the four Petri dishes were added together and divided by four to obtain the germination percentages and capacity.

The sowing of the seeds in the open field experiment was made on 23th April, which was distributed in 1 sq m parcels, between the parcels a 70 cm width were measured. On each 1 sq m parcel 40 g of seeds were sowed, from the selected grass species, varieties and grass mixtures. The experimental site was covered with commercially frost foil. The covering helped the seeds to germinate faster and also protects the seeds from leaching in case of heavy rains, the covered area was aerated one day a week. The covering was kept in place until the area had started to green up. After sowing, the experimental field was watered twice/day in the early morning and evening with 10 mm/sq m for three weeks, after the covering was removed. In May we have fertilized the area with PlantAktiv 2% solution. The first mowing was done according, when the grass reached 10 cm in height. After that the grass was mowed every 2-3 weeks at a height of 4 cm.

Height (cm) and width (cm) was determined of the selected different grass species, varieties and grass mixtures, 20 grass stems per replicate, resulting in 80 measurements per species/variety.

Density was determined using a 5 × 5 cm diameter square, which was fixed by poking it into the grass and cutting the inside out below root depth using a scalpel. After cutting, was obtained a 5 × 5 cm clump of grass, which was counted one by one; 4 samples per replicate to get a total of 16 grass clumps.

To determine the susceptibility to rust disease, were used 1 × 1 meter cardboard sheet on which 10 squares of 10 × 10 cm diameter were cut out. The cardboard sheet was placed on the grass and graded the exposed grass where the squares were cut out, according to its susceptibility to rust disease, from 0 to 5. Where 0 meant no disease tendency and 5 meant fully infested for the 10 × 10 cm square.

In order to determine the trampling tolerance an 80 × 80 cm wood board, which was placed on the top of the grass surface and two people stamp on it for 5 minutes. The trampling was performed on one replicate of each variety and mixture. After that, was observed daily when the grass surface regained its original shape.

The color of grass species within species was determined using the coloroid color system (Nemcsics, 2000). The samples were collected and then coded by using the color system.

Softness was determined by palpation. Ten people were selected to rate the grass species and mixtures within each species from 0 to 5 for softness per plot. A bonitation score of 0 meant harshest grass and a score of 5 meant softest.

The significance of the differences between the treatments was tested by applying one-way ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$.

Bars in Figures 4 and 5 represent the means ± SE ($n = 20$), furthermore in Figures 6, 7, 8, and 9 represent the means ± SE ($n = 4$). Different letters indicate significant differences between treatments ($p < 0.05$).

RESULTS AND DISCUSSIONS

Determining the germination of the seeds is important factor for sowing, because in case of low germination percentage, a larger amount of grass seeds should be used. From our results could be determined that the highest germination percentage (Figure 3a) was recorded at FaT (95.75%) and on contrary the lowest germination percentage at PpB (21.5%). At *Festuca* genus the germination percentage were almost similar at all species and varieties, however the FrrL recorded a lower germination percentage of 78%, on the other hand FaT recorded the highest. At the *Festuca* genus the average germination percentage was 86.82%. Furthermore, in the ca of *Poa* genus the germination percentage at the PpA (39.75%) and PpB (21.5) was lower compared to the other species, yet the PpE recorded the highest percentage with 82% of germinated seeds. Regarding, the lawn mixture the highest germination was determined at Reg (93%), followed by Sun (84%), Sha (70.5%), and the lowest recorded percentage was at Spo (66.75%). Considering the germination capacity (Figure 3b), here again the greatest was observed at FaT (98%) and the smallest at PpB (45.25%). *Festuca* genus recorded a 92% of average germination capacity, in which case the lowest was at FrrL. Moreover, *Poa* genus reported an average germination capacity of 72% and the lawn mixture an 89.88%. According to the Romanian standard, (1999) the germination time for *Festuca arundinacea* is 14 days, for *Festuca rubra* 21 days, and for *Poa pratensis* 28 days.

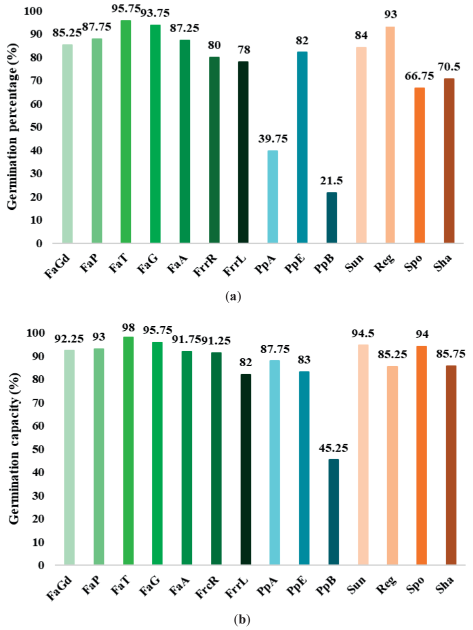

Figure 3. Germination percentage (**a**) and capacity (**b**) of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

Considering the height of the selected grass species, varieties and grass mixtures could be clearly observed that statistically significant differences were present among them (Figure 4). In this case the species and varieties height were determined, because the mixtures contained the similar seeds, however in different percentages. The FaT, FaG, FaA, FrrL and PpA recorded significant increases compared to the other grass seeds. At *Festuca* genus FaGd, Fap and FrcR reported a smaller growth compared to the other species/varieties. Regarding the *Poa* genus statistically significant changes were determined at PpB compared to the other two varieties, moreover this variety obtained the smallest growth.

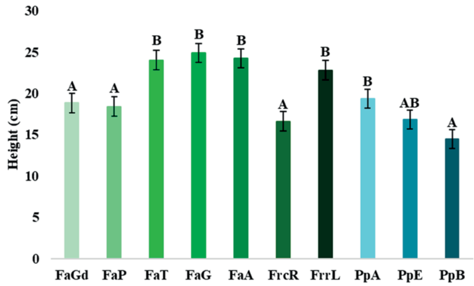

Figure 4. Growth (cm) of the of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

From our data, can be determined that, in most of the case the *Festuca* genus reported wider blade of grass than the *Poa*. However, FrcR and FrrL were significantly slimmer than the other species from this genus. No significant differences were observed at the *Poa* genus.
Under our experimental conditions, statistically significant differences were determined in the case of the density (pcs) when the selected grass species, varieties and grass mixtures were compared (Figure 6). The highest values were recorded at FrcR and the thinnest density at FaA. When comparing the selected species and varieties from the *Festuca* genus, significant increases were observed at FrcR and FrrL compared to the others. However, in the case of FaGd, FaP, FaT, FaG, and FaA no significant differences were observed.
Regarding, the *Poa* genus statistically significant differences were observed only in the case

of PpB compared to the other two varieties (PpA and PpE). Furthermore, no significant changes were determined between the lawn mixture.

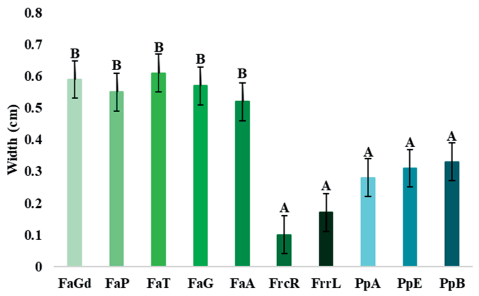

Figure 5. Width (cm) of the of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

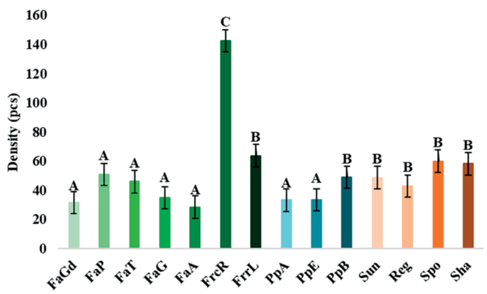

Figure 6. Density (pcs) of the of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

From the obtained results, could be clearly determined that, statistically significant differences were observed between the selected grass species, varieties and mixtures (Figure 7). The lowest bonitation points were reported at FaG (1), on the other hand the highest points were gathered from FrcR. When comparing the species and varieties from the *Festuca* genus, between FaGd, FaP, FaT, and FaA no significant differences were determined, however at FaG, FrcR, and FrrL statistically significant changes were recorded compared to the others. Regarding the *Poa* genus no significant differences were observed. Furthermore, at the lawn mixtures, here again no statistically significant changes were determined.

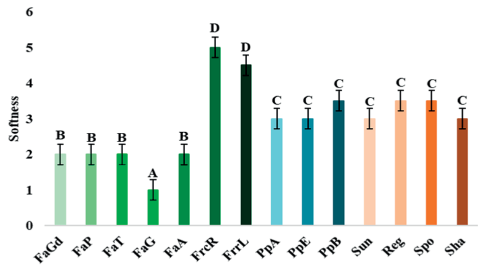

Figure 7. Softness of the of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

One of the important characteristics of grass varieties is their colour and to distinguish them more easily it is necessary to identify them (Table 2).

Table 2. Colour identification of grass species using the Coloroid colour system

Species and varieties	Coloroid cod
Festuca arundinacea 'Green dwarf' (FaGd)	A73, 15/45
Festuca arundinacea 'Patron' (FaP)	A73, 10/45
Festuca arundinacea 'Titanium' (FaT)	A73, 15/45
Festuca arundinacea 'Greystone' (FaG)	A73, 20/55
Festuca arundinacea 'Asterix' (FaA)	A73, 20/55
Festuca rubra commutata 'Rushmore' (FrcR)	A74, 20/50
Festuca rubra rubra 'Livision' (FrrL)	A73, 30/60
Poa pratensis 'Arrowhead' (PpA)	A72, 20/60
Poa pratensis 'Evora' (PpE)	A72, 25/55
Poa pratensis 'Baron' (PpB)	A73, 30/60

In the present experiment we have used the Coloroid colour atlas by Antal Nemcsics to determine the colour of the varieties. The colours correspond to codes in the colour atlas. It is mentioned that the colour of the grass in the most part is influenced by the species and variety composition, yet the soil, water, nutrients availability, weather and light conditions and even diseases can have affect (Głąb et al., 2020). The rust diseases appearance on the selected grass species, varieties and lawn mixtures was determined during the experiment (Figure 8). The highest diseases appearance was observed at PpA and PpE. On the other hand, the most resistant varieties were the FrcR and FrrL. When comparing the species and varieties from the *Festuca* genus, the highest infection with rust disease was determined at FaG and the lowest at FrcR and FrrL. The varieties belonging to *Poa* genus were the mostly infected by the rust disease. Regarding the lawn mixtures the highest diseases index was observed at Spo, which was statistically significantly higher, compared to the other three lawn mixtures.

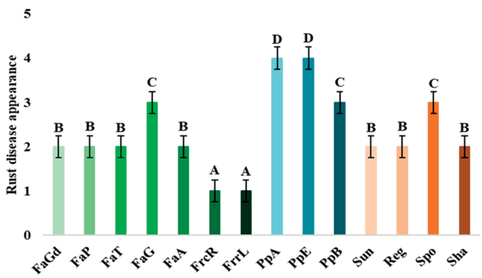

Figure 8. Rust disease appearance of the of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

Under our experimental conditions, the highest fresh mass was measured at the FaG, which was statistically significantly higher compared to the others (Figure 9). On the contrary the smallest fresh-green mass was determined at the varieties belonging to the *Poa* genus.

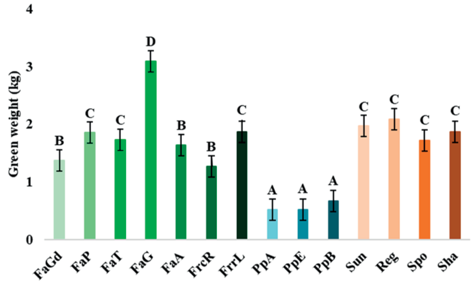

Figure 9. Fresh-green weight of the of the selected grass species, varieties and grass mixtures. *Festuca arundinacea* 'Green dwarf' (FaGd), *Festuca arundinacea* 'Patron' (FaP), *Festuca arundinacea* 'Titanium' (FaT), *Festuca arundinacea* 'Greystone' (FaG), *Festuca arundinacea* 'Asterix' (FaA), *Festuca rubra commutata* 'Rushmore' (FrcR), *Festuca rubra rubra* 'Livision' (FrrL), *Poa pratensis* 'Arrowhead' (PpA), *Poa pratensis* 'Evora' (PpE), *Poa pratensis* 'Baron' (PpB), full sunshine lawn (Sun), regenerative lawn (Reg), sports field lawn (Spo) and shady place lawn (Sha)

The varieties of the different genera recovered their original form after different or equal periods of time (Table 3). The FaGd, FaP, and FaT varieties in the table regained their original shape the day after trampling. PpE regained its shape two days after trampling. From the observations after the third day the PpA, PpB, and mixtures Spo and Sha regained their shape. Varieties FaG, FaA, and Reg mixture achieved full shape recovery on the fourth day. Even the latest FrcR and the Sun mixture regained their original shape six days after trampling.

Table 3. The recovery of grass species after trampling

Species, varieties and grass mixtures	Days					
	1 day	2 days	3 days	4 days	5 days	6 days
Festuca arundinacea 'Green dwarf' (FaGd)	1	1	1	1	1	1
Festuca arundinacea 'Patron' (FaP)	1	1	1	1	1	1
Festuca arundinacea 'Titanium' (FaT)	1	1	1	1	1	1
Festuca arundinacea 'Greystone' (FaG)	2	2	2	1	1	1
Festuca arundinacea 'Asterix' (FaA)	2	2	2	1	1	1
Festuca rubra commutata 'Rushmore' (FrcR)	3	2	2	2	2	1
Festuca rubra rubra 'Livision' (FrrL)	3	2	2	1	1	1
Poa pratensis 'Arrowhead' (PpA)	2	2	1	1	1	1
Poa pratensis 'Evora' (PpE)	2	1	1	1	1	1
Poa pratensis 'Baron' (PpB)	2	2	1	1	1	1
Full sunshine lawn (Sun)	3	2	2	2	2	1
Regenerative lawn (Reg)	2	2	2	1	1	1
Sports field lawn (Spo)	3	2	1	1	1	1
Shady places lawn (Sha)	3	2	1	1	1	1

1 - regenerative, 2 - intermediate, 3 - non-regenerative

These present study results are partly explained by the fact that *Festuca rubra* and *Poa pratensis* are the "weakest" in terms of plant height and fresh weight, and that these two species are also tracked bedstraws, which germinate and sprout more slowly, are shorter but have a longer life span. On contrary *Festuca arundinacea* is a loosestrife that germinates and develops quickly, has greater height and vigour, but a shorter life span. In a previous study conducted that the 'Landscaper Pro Rapid' (*Festuca rubra* 'Cathrine' 15%, *Lolium perenne* 'Vermino' 40%, *Lolium perenne* 'Groundforce' 35%, *Poa pratensis* 'Heatmaster' 10%) resulted the best germination percentage (Buru et al., 2021). In a study is mentioned that returning the cut grass to the mowed area, has a beneficial impact on the colour and height (Knot et al., 2017).

CONCLUSIONS

The present experiment provides data on the comparison of different species, varieties and grass mixtures. According to the obtained results, it can be concluded that at the highest germination percentage and capacity was recorded at FaT. Regarding the height FaT, FaG, FaA, FrrL, and PpA obtained the greatest data, furthermore in the case of width FaGd, FaP, FaT, FaG, and FaA varieties. Considering the density, from the present results, could be concluded that the densest grass was the FrcR. In the case of the softness, again the FrcR obtained the most points. Moreover, at rust disease appearance, the most resistant were the FrcR and FrrL grass varieties. Regarding the fresh green mass, the lowest weight was determined at the *Poa* genus, which in some case would be a good thing, expect that the grass main reason is to cover the soil, and bring a little bit of nature into our outdoor relaxing places. All together we can conclude that the FrcR is the most preferable, because of a good density, softness, and it is rust resistant.

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MORPHOLOGICAL AND PHYSIOLOGICAL PARTICULARITIES OF FIVE DAHLIA CULTIVARS

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Abstract

The paper presents the results of the morphological characteristics of Dahlia leaves (leaves area, the perimeter, the length of the leaves, width of leaf), physiological processes (photosynthesis, respiration and transpiration) and chlorophyll content determined at 5 Dahlia cultivars 'Topmix Red', 'Hy Pimento', 'Babylon Red', 'Marble Ball' and 'Thomas Edison'. The average leaf area has varied from 5.35 cm^2 ('Topmix Red') to 68.41 cm^2 ('Babylon Red'). As for the perimeter, 'Babylon Red' had the highest value (67.49 cm) and 'Topmix Red' had the lowest value (9.72 cm). The length of the leaves was between 14.19 cm for 'Babylon Red' and 4.43 cm for 'Topmix Red'. The width of the leaves was between 10,18 cm for 'Babylon Red' variant and 2.26 cm for 'Topmix Red'.The intensity of photosynthesis varied between 12.85 µmol CO_2 m^{-2}s^{-1} for 'Babylon Red' and 15.24 µmol CO_2 m^{-2}s^{-1} for 'Topmix Red', intensity of transpiration 4.69 mmol H_2O m^{-2}s^{-1} for 'Hy Pimento' and 6.17 mmol H_2O m^{-2}s^{-1} for 'Thomas Edison', intensity respiration 7.46 µmol CO_2 m^{-2}s^{-1} for 'Hy Pimento' and 13.43 µmol CO_2 m^{-2}s^{-1} to 'Marble Ball'. The total chlorophyll content was higher in the case of 'Marble Ball', at a value of 163.90 mg/100g, and the smallest quantity was recorded for the 'Babylon Red' at the value of 102.15 mg/100g.

***Key words**: chlorophyll, leaf morphology, photosynthesis, respiration, transpiration.*

INTRODUCTION

The special floricultural qualities of the species of the genus Dahlia make these plants not lacking in gardens and green spaces. The genus Dahlia is distinguished by 42 species and multitude of varieties as different as possible (http://www.theplantlist.org/1.1/browse/A/Compositae/Dahlia/). They are easy to grow, the decoration period is quite long, and the flowers are numerous and with an impressive colour. The nutritional and medicinal properties (Mejía et al., 1992; Laguna, 1992) of these plants have been known since Aztec times, and all these uses have led to the study of Dahlia species and varieties by researchers around the world (Ovando et al., 2006). Thus, numerous morpho-anatomical, physiological, nutritional and medicinal research with a great scientific value have been carried out, which confirms that these plants bring benefits to humans (Ciobanu (Țurlea) et al., 2021; Mejía et al., 1992; Laguna, 1992, https://www.sarahraven.com/articles/history-of-the-dahlia.htm).

In recent decades, the food industry has focused on the search for potential sources of anthocyanins that are able to provide color to replace synthetic dyes and at the same time provide health benefits through food products. The ancestral use of the flower in several dishes, its abundance, and the intense color of the flowers known as black make the *Dahlia pinnata* flower a suitable candidate to be considered as a potential source of anthocyanins (Granados-Balbuena, 2022). Dahlia belongs to Compositae (Asteraceae family), which is the largest family, comprising over 32913 species worldwide (http://www.theplantlist.org/1.1/browse/A/Compositae/). Dalia has worldwide fans because it is a plant that easily adapts to environmental conditions and because of the great variety of species, thus providing satisfaction to anyone who wants to cultivate it (Cantor & Pop, 2008).

Dahlias grew and still grow like weeds in the mountainous regions of Mexico and Central America and the Aztecs used them for food (Santana et al., 2016).
Eid et al. (2011) mention that there are over 2000 named cultivars of *Dahlia variabilis* in the USA. The natural geographic range for the genus Dahlia includes the natural Sierra Madre Occidental region of Mexico (Figure 1). Exceptions are *Dahlia australis*, which occurs at least as far south as southwestern Guatemala, and *Dahlia coccinea* and *Dahlia imperialis*, which have been reported throughout Central America into northern South America. Plant size varies from the small *Dahlia tenuis* and *Dahlia scapigera*, which only average 30-60 cm in height on slender stems, to tall arborescent species such as *Dahlia tenuicaulis*, to *Dahlia macdougallii*, which grows from under the mosses and ferns covering tree trunks and produces long shoots that sprawl across the canopy branches of tropical hardwoods.

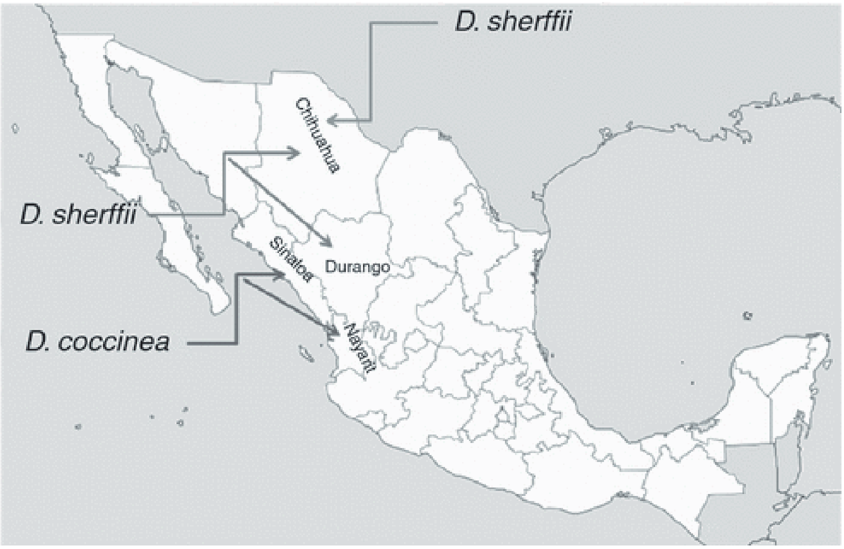


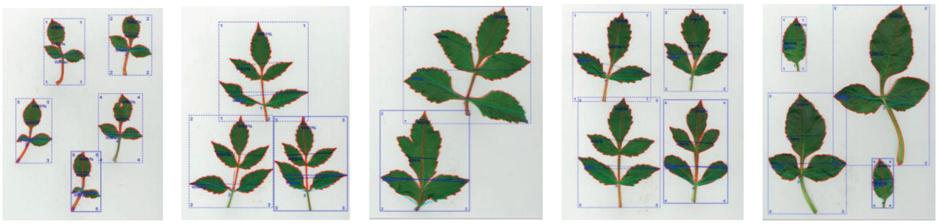
Figure 1. Map of Mexico showing the occurrence of the four wild *Dahlia* spp. Samples were collected from Nayarit, Sinaloa, Chihuahua and Durango states.
Source: https://bsppjournals.onlinelibrary.wiley.com/doi/10.1111/j.1365-3059.2010.02367.x

Selected wild Dahlia species in their natural habitats from west-central Mexico were tested for the presence of three caulimoviruses known to be associated with cultivated dahlia (*Dahlia variabilis*), viz. Dahlia mosaic virus (DMV), DMV-D10 and Dahlia common mosaic virus. The discovery of plant pararetroviruses in wild dahlia species in their natural habitats suggests a possible emergence, co-existence and co-evolution of pararetroviruses and their host plants (Eid et al., 2011).
The dahlia crop is easy to maintain; dahlias are recognized for their ornamental, nutritional, pharmaceutical and antibacterial properties, and researchers want to identify their morpho-physiological traits according to the action of environmental factors in order to obtain the best possible production.

MATERIALS AND METHODS

The plants taken into study were cultivated in the "I. Todor" Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The morphological and physiological characteristics were analysed in 5 cultivars of *Dahlia variabilis*: ‘Topmix Red’, ‘Hy Pimento’, ‘Babylon Red’, ‘Marble Ball’ and ‘Thomas Edison’. To determine leaf morphological characteristics, all leaves of one plant were harvested from each of the 5 Dahlia cultivars in July 2022, in the seventh decade from planting. The leaves were washed with water, dried and scanned using an Epson Expression 11000XL scanner and then analyzed with software the WinFolia. The determinations done are the leaf area (cm^2), the perimeter (cm), the length (cm) and the width (cm) of the leaves (Figure 2). The determination of the intensity of photosynthesis, transpiration and respiration was performed directly on the green part of leaves, in the field of experience, using the LCPro+ equipment, according to Aelenei et al. (2020). The determinations for the leaves of Dahlia plants were made with the LCPro+ equipment (according to Lascu et al. (2019)) in a relatively small range of variation of the intensity of photosynthetic active radiation of 1458-1484 µmol m^{-2}s^{-1}, at temperature 39.1-42°C (Table 2). Triplicates of independent determination were reported.

The quantitative analysis of the assimilating pigments was performed through the Arnon spectrophotometric method, which is based on the extraction of pigments in an organic solvent (80% acetone) and measuring the absorbance of the extract, by reading the sample extinction at a spectrophotometer at three different wavelengths: 470 nm, 646 nm and 663 nm, according to Asănică et al. (2017).



‘Topmix Red’ ‘Hy Pimento’ ‘Babylon Red’ ‘Marble Ball’ ‘Thomas Edison’

Figure 2. Morphological traits of the plant leaves for Dahlia ‘Topmix Red’, ‘Hy Pimento’, ‘Babylon Red’, ‘Marble Ball’și ‘Thomas Edison’
Source: original

Independent extract solutions were analyzed in triplicate. Statistical data processing was done by Microsoft Office Excel for Mac, version 16.69.1 (23011600).

RESULTS AND DISCUSSIONS

From the data regarding the biometric measurements presented in Table 1 and Figure 3 it can be observed that the average leaf area has varied from 5.35 cm (V_1 - 'Topmix Red') to 68.41 cm (V_3 - 'Babylon Red').

As for the perimeter, the variant V3 had the highest value (67.49 cm) and V1 had the lowest value (9.72 cm), mainly correlated with leaf width. The length of the leaves was between 14.19 cm for V3 and 4.43 cm for V1.

Table 1. Biometrical measurements of the leaves

Var.	Cultivar	Leaf Area (cm^2)	Perimeter (cm)	Lenght (cm)	Width (cm)
V_1	'Topmix Red'	5.35 ± 0.41	9.72 ± 0.68	4.43 ± 0.21	2.26 ± 0.11
V_2	'Hi Pimento'	40.66 ± 5.54	63.84 ± 6.98	12.04 ± 0.9	8.61 ± 0.8
V_3	'Babylon Red'	68.41 ± 7.5	67.49 ± 7.54	14.19 ± 0.99	10.18 ± 0.9
V_4	'Marble Ball'	26.75 ± 2.02	39.9 ± 2.6	9.26 ± 0.36	6.76 ± 0.4
V_5	'Thomas Edison'	44.28 ± 3.44	29.58 ± 2.06	12.4 ± 0.66	6.27 ± 0.35

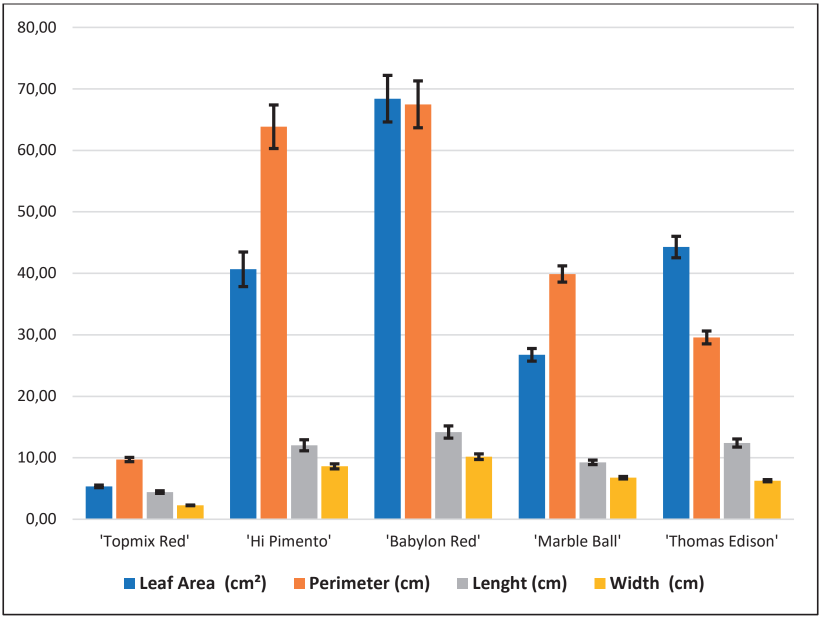


Figure 3. Biometrical measurements of the leaves (cm^2/cm)
Source: original

The width of the leaves was between 10.18 cm for V3 variant and 2.26 cm for V_1. The perimeter and the width showed the highest values in the V3 variant and the smallest in the V_1 variant.

In these conditions, it was registered different degrees of stomatal opening (between 0.18 - V_2 and 0.25 - V_5) that influenced physiological parameters.

Table 2. Intensity of photosynthesis, transpiration and respiration of Dahlia leaves, Q leaf - intensity of light incident on leaf, T°C - leaf temperature

Var.	Cultivar	Light intensity on leaf ($\mu mol^{-2}s^{-1}$)	Degree of stomatal opening (mol H_2O $m^{-2}s^{-1}$)	Leaf temp. (°C)	Photosynthesis (μmol CO_2 $m^{-2}s^{-1}$)	Transpiration (mmol H_2O $m^{-2}s^{-1}$)	Respiration (μmol CO_2 $m^{-2}s^{-1}$)
V_1	'Topmix Red'	1483	0.23	39.16	15.24	4.81	7.84
V_2	'Hy Pimento'	1484	0.18	39.50	12.88	4.69	7.46
V_3	'Babylon Red'	1473	0.19	40.71	12.85	5.19	11.52
V_4	'Marble Ball'	1475	0.24	41.05	13.95	6.07	13.43
V_5	'Thomas Edison'	1458	0.25	42.00	12.92	6.17	12.91

The intensity of the photosynthesis process ranged from 12.85 µmol CO_2 $m^{-2}s^{-1}$ for 'Babylon Red' to 15.24 µmol CO_2 $m^{-2}s^{-1}$ for 'Topmix Red'. The leaf surface and the position of the leaves against the solar radiation are important factors that condition the intensity of the photosynthesis process (Burzo et al., 2004).

Regarding the intensity of the transpiration process according to the data presented in Table 2, the variation limits were between 4.69 mmol H_2O $m^{-2}s^{-1}$ for 'Hy Pimento' and 6.17 mmol H_2O $m^{-2}s^{-1}$ for 'Thomas Edison'. The intensity of transpiration varies with the species, the age of the plant and the environmental conditions (Toma & Jităreanu, 2007). The intensity of physiological processes varies according to the characteristics of the species and due to the climatic conditions. At the flowering plants, the intensity of photosynthesis is particularly influenced by light intensity and temperature. Determinations made at Dahlia show that the light intensity of 1.778 µmol $m^{-2}s^{-1}$ has a photosynthesis intensity of 11.67 µmol CO_2 $m^{-2}s^{-1}$. With regard to the process of transpiration, research conducted at a temperature of 34.4°C indicates the intensity of transpiration of 5.75 μmol H_2O $m^{-2}s^{-1}$ (Burzo et al., 2000).

From the measured data, a weak negative correlation was recorded between the intensity of photosynthesis and the intensity of transpiration - Figure 4 - (R^2 = 0.0499, y = - 0.2446x + 15.136, where y = intensity of photosynthesis and x = intensity of transpiration) probably according to the degree of stomatal opening and the temperature at the level of the leaf; it is inverse correlation, the two correlated variables vary in the opposite direction (when one increases, the other decreases).

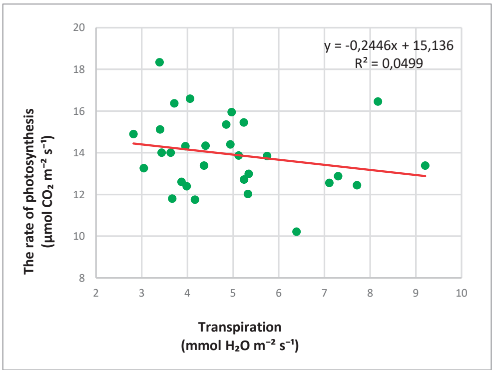


Figure 4. The correlation between the rate of photosynthesis and the transpiration at the leaves of Dahlia cultivars
Source: original

The intensity of physiological processes depends on the temperature (Nicolae, 2010). Light intensity and temperature are primary environmental factors affecting the growth and development of plants. Plants have evolved several efficient protective mechanisms that make it possible for them to survive under unfavorable light and temperature conditions. These mechanisms are linked predominantly to the photosynthetic electron transport chain, xanthophyll cycle and the photorespiratory pathway. Under stress conditions, elevated levels of reactive oxygen species (ROS) are produced, which in addition to deleterious effects also show signaling functions. Under severe, short light/temperature stress, the contents of low-molecular weight antioxidants, such as ascorbate, glutathione and prenyllipids, tend to decrease, which is correlated with an extra need for ROS scavenging. Under longer exposure of plants to unfavorable light and temperature conditions, the contents of antioxidants gradually increase as a result of acclimation during long-term responses (Szymańska et al., 2017). In their natural environment, plants are exposed to various stressors that act together, and intense irradiations combined with increased temperatures are the most frequently experienced stresses under field conditions. When changes in environmental conditions exceed plant capacity for acclimation, photoinhibition occurs (Nishiyama et al., 2011).

Linear regression made between the rate of photosynthesis and the leaf temperature shows a perfect inverse (negative) correlation between the two analyzed factors; the coefficient of determination (R^2) was 0.3382, y = - 0,52x + 34,977, where y = intensity of photosynthesis and x = temperature (Figure 5).

Nicolae (2010) mentions that the linear regression between photosynthesis rate and leaf temperature shows a good correlation; the coefficient of determination (R^2) was 0.83 in *Dahlia variabilis* for the following temperature values: at 28.4°C, the rate of photosynthesis was 1.105 μmol/m^2/s, at 33.8°C, the rate of photosynthesis was 1.410 μmol /m^2/s, and at 29.8°C, the rate of photosynthesis recorded the value of 1.280 μmol/m^2/s.

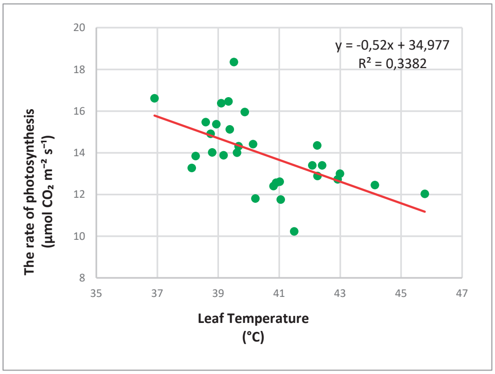


Figure 5. The correlation between the rate of photosynthesis and the temperature at the leaves of *Dahlia* cultivars
Source: original

Environmental temperature not only influences physiological processes, but also the growth of dahlia plants. Schneck et al. (2021), notes that the supraoptimal root-zone temperatures (RZTs) to *Dahlia* x *hybrida* during production may decrease dahlia root quality, especially above 40°C and could initiate dahlia decline.

Overall plant height was significantly impacted, resulting in shorter heights to plants under the influence of treatments - the supraoptimal root-zone temperatures between 40°-50°C.

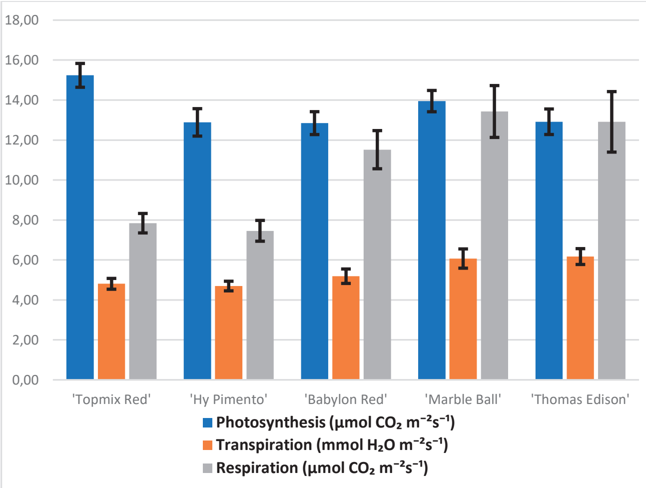


Figure 6. Intensity of the physiological processes
Source: original

Regarding the intensity of the respiration process (Figure 6) it can be appreciate that the lowest value of the intensity of the respiration was recorded for V_2 ('Hy Pimento'), 7.46 µmol CO_2 $m^{-2}s^{-1}$, and the highest value was recorded for V_4 ('Marble Ball'), 13.43 µmol CO_2 $m^{-2}s^{-1}$.
The high values of the respiration process are due to the high temperatures at which the determinations were made. For 'Babylon Red', 'Marble Ball', 'Thomas Edison' the temperature exceeded the critical value of 40°C, which led to a decrease in the intensity of photosynthesis and an increase in respiration and transpiration. Also, at these high temperatures, the stomata are wide open to allow the exit of water vapor through the ostioles and at the same time the elimination of excess heat along with the vaporization of water and the achievement of transpiration.
Considering the high temperature at which the determinations were made, the increase in respiration can also be attributed to the increase in photorespiration, knowing that the high temperature favors the oxidizing function of the RUBISCO enzyme and implicitly the biodegradation of photoassimilates in the photorespiration process. In the five Dahlia cultivars studied, the temperatures above 40°C negatively affect the photosynthesis process, positively stimulate transpiration (due to the thermoregulatory role), photorespiration and respiration.
Determinations regarding the content of chlorophyll and carotenoid pigments in the species of the Dahlia genus led to the results presented in Table 3. They highlighted the modification of the content of assimilating pigments according to the species, as follows: the chlorophyll a content was higher in the case of 'Marble Ball', at a value of 97.66 mg/100 g, and the smallest quantity was recorded for the V_3 - 'Babylon Red' at the value of 77.07 mg/100g, according to Figure 7. The quantity of chlorophyll b ranged from 25.07 mg/100g in V_3 ('Babylon Red') to 66.23 mg/100g in V_4 ('Marble Ball').
It should be noted the increased content of chlorophyll and carotenoid pigments in V_1 ('Topmix Red') in positive correlation with the photosynthesis rate.

Table 3. Assimilatory pigments content

Var.	Cultivar	Chl. a (mg/100 g)	Chl. b (mg/100 g)	Total Chlorophyll (mg/100 g)	Carotenoids (mg/100 g fw)	Chl. a/Chl. b ratio
V_1	'Topmix Red'	88.76	41.79	130.55	15.60	2.13
V_2	'Hi Pimento'	77.47	29.40	106.87	10.95	3.00
V_3	'Babylon Red'	77.07	25.07	102.15	10.16	3.55
V_4	'Marble Ball'	97.66	66.23	163.90	13.63	1.54
V_5	'Thomas Edison'	75.87	28.89	104.76	10.09	3.49

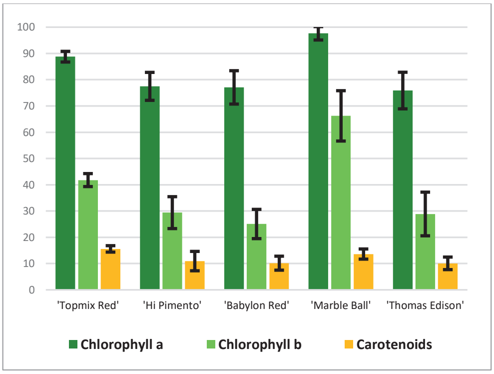


Figure 7. Content of chlorophyll and carotenoid pigments (mg/100 g)

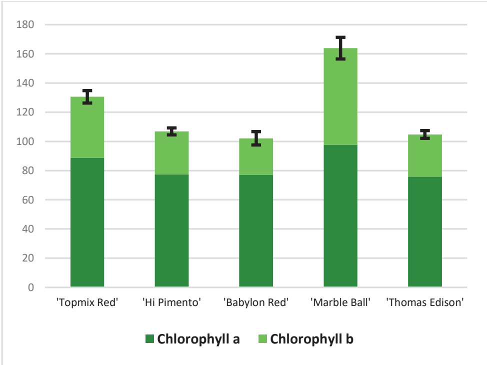


Figure 8. Content of total chlorophyll (mg/100 g)

The total chlorophyll content was higher in the case of V_4 - 'Marble Ball', at a value of 163.90 mg/100 g, and the smallest quantity was recorded for the V_3 - 'Babylon Red' at the value of 102.15 mg/100 g, according to Table 3, Figure 7 and 8.

The plants of *Dahlia variabilis* watered in the controlled way had a higher content of chlorophyll and carotenoids than the plants watered in the standard way (Jedrzejuk et al., 2022).

It seems that in the present research, the high total chlorophyll content is influenced by the culture conditions considering that the dahlia culture was irrigated daily (morning) for 2 hours (drip system).

Linear regression made between the rate of photosynthesis and of the total chlorophyll shows a weak correlation between the two analyzed factors; the coefficient of determination (R^2) was 0.0536, y = 0,0166x + 11,914, where y = intensity of photosynthesis and x = total chlorophyll (Figure 9).

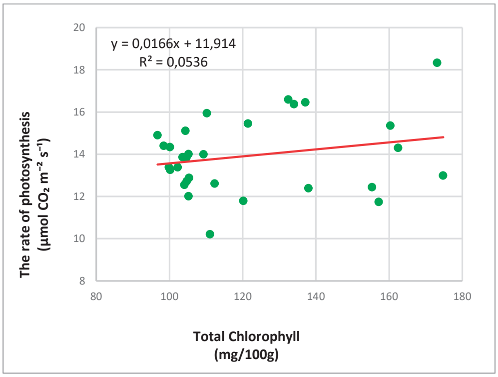


Figure 9. The correlation between the rate of photosynthesis and the total chlorophyll at the leaves of Dahlia cultivars

Source: original

A weak negative correlation was recorded between the intensity of photosynthesis and the leaf area - Figure 10 - (R^2 = 0.0816, y = -0.0235x + 14.797, where y = intensity of photosynthesis and x = intensity of transpiration).

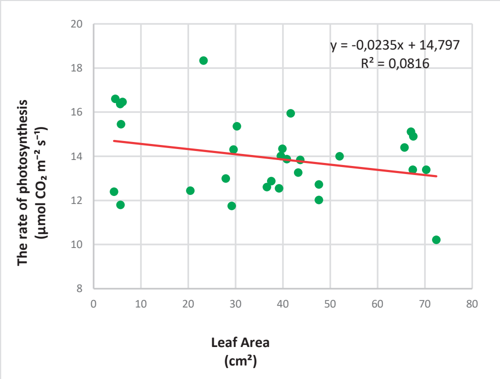


Figure 10. The correlation between the rate of photosynthesis and the leaf area at the leaves of Dahlia cultivars

CONCLUSIONS

The results obtained varied according to the cultivars and the assimilatory pigments distribution in leaf, which positively influenced the photosynthesis in leaves. The ornamental plants taken into study are useful resources for further research regarding the morphological aspects correlated with physiological processes that are carried out at the leaf level. The average leaf area has varied from 5.35 cm (V1 - 'Topmix Red') to 68.41 cm (V3 - 'Babylon Red'). The intensity of the photosynthesis process ranged from 12.85 µmol CO_2 $m^{-2}s^{-1}$for 'Babylon Red' to 15.24 µmol CO_2 $m^{-2}s^{-1}$for 'Topmix Red'. The intensity of physiological processes varies according to the climatic conditions. The total chlorophyll content was higher in the case of V4 - 'Marble Ball', at a value of 163.90 mg/100g, and the smallest quantity was recorded for the V3 - 'Babylon Red' at the value of 102.15 mg/100 g. A positive correlation was recorded between the intensity of photosynthesis and the total chlorophyll.

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THE NORWAY MAPLE APHID - *PERIPHYLLUS LYROPICTUS* (KESSLER) (*HEMIPTERA: APHIDIDAE*): A NEW PEST OF *ACER PLATANOIDES* IN WESTERN ROMANIA

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Abstract

Periphyllus lyropictus (Kessler) was collected for the first time in the green urban landscape of Timisoara, Western Romania. Small colonies with oviparous female and alate males of the Norway maple aphid were recorded on Acer platanoides trees between October and November 2022. This aphid species is also reported for the first time in Romania. The native range of the species includes Europe and Asia. The aim of this paper was to provide information on potential damage to host plants and some morphological characteristics, original images being presented. Some reference data on the biology and distribution are highlighted. Norway maple aphid populations were found in each of the 6 parks and private gardens surveyed and in 115 of the total 180 trees analyzed.

Key words*: Periphyllus lyropictus, aphid, Acer platanoides, western Romania.*

INTRODUCTION

Norway maple (*Acer platanoides*) is the most widespread native maple in Europe, with a natural distribution range from central Europe and reaching eastwards into the Ural Mountains (Caudullo & de Rigo, 2016). It occur in all types of urban habitats (having the ability to withstand many urban impacts) and is considered to be the most common and ornamental-relevant deciduous tree within the green urban-ecosystem in Europe and Romania (Nowak & Rowan, 1990; Savill, 1991; Sæbø et al., 2003; Sjöman & Nielsen, 2010).

This tree, through its great diversity of cultivars, provides refuges and corridors for the movement of invasive invertebrate species (Barczak et al., 2021), among which the invasive insect species (around 85% of invasive invertebrates in Europe) stand out (Roques et al., 2009). The threat of alien insect species, spread in urban green landscape in Romania is very real, the Norway maple not being an exception.

Once with trade expansion and the accession to the EU, customs controls were reduced, at the same time the transport of plant material increased a lot, and allowed the entry into our country of a growing number of new invasive insect species (Nețoiu et al., 2018), aphid species representing, along with: Metcalfa pruinosa (Gogan et al., 2010), Halyomorpha halys (Macavei et al., 2015), a serious threat to Norway maple trees in terms of depreciating their landscape value.

In the last period of time, in Europe, there has been an intensification of aphids attack on *Acer* sp. (*Aceraceae*) (Depa & Mroz, 2013; Mackoś-Iwaszko et al., 2015; Koranyi & Marko, 2022). Blackman & Eastop (2023) mention 96 species of aphids on Maples and Sycamores, that already have stable populations in the green urban landscapes around the world, mainly these species belonging to *Periphyllus* (35 spp.) and *Drepanaphis* (20 spp.) genera.

The genus *Periphyllus* van der Hoeven 1863 (*Hemiptera, Aphididae: Chaitophorinae*) is a Palaearctic genus with 50 species described worldwide (Blackman and Eastop, 1994; Lubiarz & Macko´s-Iwaszko, 2015), of which 14 species in Europa (Tomić & Petrović-Obradović, 2022). To date, five species of *Periphyllus* (*acericola, aceris, lyropictus, testudinaceus, coracinus*) have been recorded in the neighbouring countries: 4 species in Hungary (Ripka et al., 1998) and 4 in Serbia (Tomić & Petrović-Obradović, 2022) on *Acer platanoides*. In this study we report *Periphyllus* *lyropictus* (Kessler) for the first time in Romania, occurring on *Acer platanoides* in green urban landscapes. Morphological description, biology and distribution data, and also photograps of the specimen are presented.

MATERIALS AND METHODS

The research was carried out in the area of Timișoara (Timiș: Romania) between October and November 2022. Six green urban study sites were selected: site 1 – University of Life Science Park (45°782'599" N, 21°215'545" E); site 2 – Botanical Garden (45°760'653" N, 21°225'645" E); site 3 – Central Park Anton von Scudier (45°751'027" N, 21°221'747" E); site 4 – Cathedral Park (45°750'226" N, 21°224'386" E); site 5 – Justice Park (45°749'911" N, 21°227'497" E); site 6 – one private garden situated on C.D. Loga Boulevard (45°753'602" N, 21°237'229" E). For each site we selected 5 *Acer platanoides* trees (a total of 30 trees), from which at every 10 day, 25 leaves/ tree were collected. Samples were taken on 6 dates: 3, 11, 20, 31 October, 9 and 20 November.

After collection, the aphids, along with the leaves of the host plants, were transported to the Diagnostic and Phytosanitary Laboratory, where the specimens were photographed. Most of the aphids were preserved in 70% alcohol (Petrović-Obradović et al., 2021), only some were mounted on microscope slides using standard methods (Eastop & van Emden, 1972). The following identification keys were used to determine the aphid species: Hille Ris Lambers (1947); Blackman & Eastop (1994); and the website: www.InfluentialPoints.com.

RESULTS AND DISCUSSIONS

Periphyllus lyropictus (Kessler) was identified for the first time in the green urban landscape of Timisoara, Western Romania, and also, for the first time in Romania, in 3 October 2022 on *Acer platanoides* ornamental trees. Small colonies of larvae, oviparous females and alate males were registered on the underside of leaves.

In the survey conducted in green urban spaces between October and November 2022, a total of 36 aphid samples were collected, Norway maple aphid populations being found in each of the 6 parks and private gardens surveyed and in 115 of the total 180 trees analyzed (Figure 1), no deformations were observed on the parts of the infested plants.

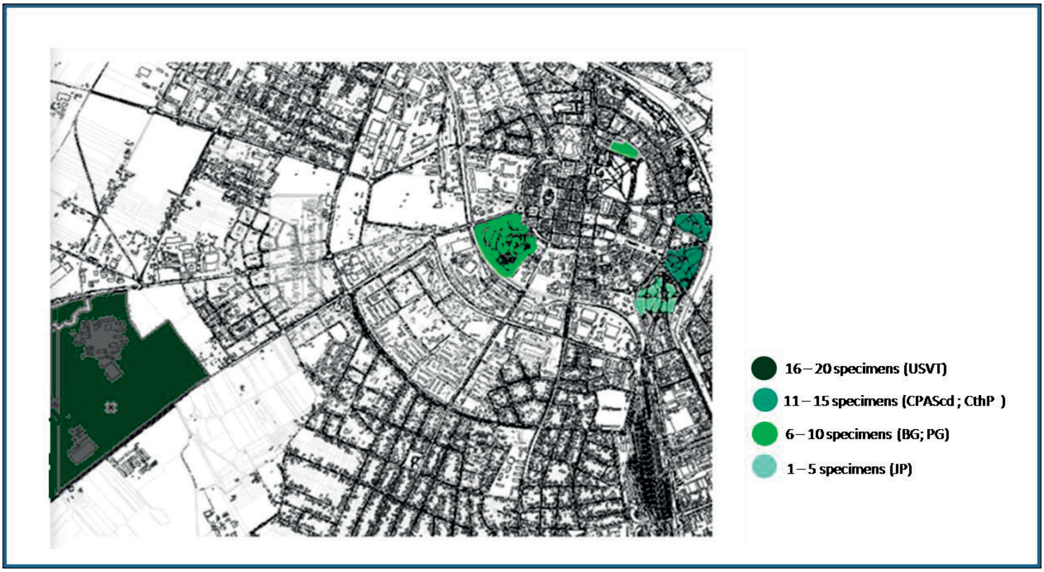

Figure 1. Collecting sites and the number of *Periphyllus lyropictus* colonies. The abbreviations denote as follow: (1) USVT - University of Life Science Park; (2) BG - Botanical Garden; (3) CPAScd - Central Park Anton von Scudier; (4) CthP - Cathedral Park; (5) JP - Justice Park; (6) PG - private garden (image modified by authors)

In 2022, the first occurrence of aphids was noted in the University of Life Sciences Park, were 3 small colonies of oviparous female, alate male and immatures were observed (Figure 2). After, in order to confirm the presence of the pests, we extended the research, investigating five more points, a total of 67 aphid colonies being reported.

Figure 2. *Periphyllus lyropictus* colony with oviparous female, alate male and immatures *on Acer platanoides* leaves in October 2022 (photo by Virteiu)

The highest number of *P. lyropictus* colonies was observed in USVT Park, 19 aphids' colonies/ 25 leaves were observed. Similar, however lower, was the number of *P. lyropictus* colonies observed in Cathedral Park, 14 aphids colonies/ 25 leaves. A significantly small number of Norway maple aphids were noted in Central Park Anton von Scudier (3 aphids' colonies).

Currently, *Periphyllus lyropictus* has only been recorded on *Acer platanoides*, although there are a few records on *Acer campestre* in Europe (Ripka et al., 1998).

The impact of this invasive pest on *Acer platanoides* in urban green spaces differs from country to country and according to the frequency of tree occurrence in landscape (Mackoś-Iwaszko et al., 2015). However, damage caused by aphids can be direct by extracting sap and preventing the flow of nutrients into the plant, affecting its metabolism, and indirect which includes covering infested leaves with honeydew excreted by the aphids. Fall generations, produced large amounts of honeydew, and in consequence, caused the decline of the decorative value of trees. Their low numbers do not seem, however, to cause any visible significant damage to maple leaves. Even though *P. lyropictus* can be classified as a species with low harmfulness, in our country future monitoring of aphid population evolution is recommended.

Oviparous females (Figure 3) are apterous, elongate oval, pale green; as it matures the body becomes darker brown. Body length: 1.9-3.25 mm. Head and thorax with a rectangular green - brownish spinal stripe and a large, dark V-shaped band on the dorsal abdomen, in front and between the siphunculi. Antennae are pale yellowish except VI and distal V which are black; the terminal process is 4.5-6.0 times as long as the base of antennal segment VI. The siphunculi are pale to dark brown, conical in shape and about as long as their basal widths. The cauda is short, tongue-shaped. The legs having the femora with brown bands, front and middle tibiae brownish, hind tibiae dilated, tarsi black (Blackman & Eastop, 2023).

Figure 3. *Periphyllus lyropictus*: oviparous female (autumn forms) on *Acer platanoides* in October 2022, Timisoara (photo by Virteiu)

The alate males (Figure 2) have a dark head and thorax, abdomen greenish to dark brown; numerous long hairs arise from the middorsal area of each abdominal segment. Antennae are black, almost the same length as the body, with a long, pale antennal segment IV and long hairs in irregular arrangement. Siphunculi are reticulated, with black colour. Cauda well rounded, as long as wide. Body length: 2.65 mm (Essig & Abernathy, 1952).

Larvae (Figure 4) are pale green to light yellow, flattened shape, with a series of very short transverse stripes in dark green to brownish along the dorsum.

Figure 4. *Periphyllus lyropictus*: larvae on *Acer platanoides* in October 2022, Timisoara (photos by Virteiu)

Periphyllus lyropictus is holocyclic and monoecious. It infests mainly species of *Acer* genus. In Romania, it has only been found on *A. platanoides*. Oviparae female and alate males are produced in October-November (this is the only forms found on leaves in our research)

CONCLUSIONS

Periphyllus lyropictus is a possible threat to Norway maples trees in the green urban landscape of Romania, as well as in the western part of the country. Aphid development was recorded exclusively on *Acer platanoides* in our observations, but it could also attack *A. campestre* and *A. pseudoplatanus*, which are important ornamental trees in urban green areas. Therefore, further surveys are needed to determine the wider distribution of the pest, the new host plants and the damage to these plants.

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***https://influentialpoints.com/Gallery/Periphyllus_lyropictus.htm

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PRELIMINARY STUDY ON THE FRUIT MORPHOLOGY, AGRONOMIC, AND PHYSIO-CHEMICAL CHARACTERISTICS OF TOMATO VARIETIES IN GREENHOUSE CONDITIONS

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Abstract

Greenhouses are now one of the most effective industries in agricultural structure, used to preserve a monitored environment suitable for crop management throughout the year. The demand for tomato products is increasing year after year, providing ample opportunities for the tomato market to grow. The variety of tomato genotypes provides a gene pool for future breeding, because they may contain traits useful for the development of new varieties and hybrids with improved quantitative and qualitative characteristics. The study aimed to evaluate the performance of five indeterminate genotypes of tomato to determine their performance under greenhouse conditions. The research was conducted utilizing biological material from the Vegetable Research and Development Station in Bacau. The data collected were: number of days till appearance of the first flowers, number of days till first fruit development, date of first harvest, as well as the outer color of the immature and ripe fruit. The results of investigations on phenotype characteristics such as fruit weight, height, diameter, dry matter content, TSS, water, minerals, and titratable acidity are discussed in the paper.

Key words*: greenhouse tomato quality, growing conditions, genotypes, local cultivar, solanaceous.*

INTRODUCTION

The agriculture industry is expected to go through a number of changes in the upcoming years. As a result of climate change, temperatures are expected to rise, but so is the price of energy. Growing crops in greenhouses requires a lot of natural resources. Any modification to the ecosystem caused by a shift in the climate system would alter the distribution of water and the patterns of precipitation.

According to official statistics from FAO in 2021, on an area of 18.130 ha, Romania produced 500.200 tons of tomatoes, and had an average production of 27.58 tons/ha (FAOSTAT, 2021). The interest in consuming high quality fresh or processed tomatoes continues to increase. In Romania, the annual net average of tomato consumption per capita was recorded in 2021 as 45.1 kg/inhabitant (INS, 2022),which is a relevant indicator for the vegetable market.

Due to its geographic location, Romania provides favorable conditions for growing extra-early vegetable crops in greenhouses since, from December to February, it experiences higher levels of light radiation than Western European nations. Following Bucharest (Popeşti greenhouses 142 ha), the largest greenhouse fields, distribution patterns, and production specializations were discovered in Arad, Timiș, Bihor, and Dolj. Additionally, zone I (Bihor, Arad, and Timiș counties) and the entire southern region of Romania were home to the most advantageous regions for the protected cultures (areas coated in various forms of plastic) (Drăghici et al., 2021; Popa et al., 2021).

Greenhouse farming is a technology that is widely used in Europe to promote efforts to achieve food security, is the technique of regulating the environmental factors for the benefit of the plant under protective cultivation. Greenhouses offer a dependable option to growing high-quality tomatoes both in and out of season by providing the ideal climatic conditions necessary for their best development and output.

To cultivate tomatoes in the greenhouse, it is necessary to take into account three important factors: the microclimate within the greenhouse (light and temperature), the cultivar being used, and the unique cultivation technology. The benefits of the technology used to grow tomatoes in greenhouses include the improved quality of the tomatoes, the staggered harvesting of the yield, the extension of the plants' autumn vegetative season, and the avoidance of hail and torrential rain-related risks (Inculet & Stoleru, 2021).

The tomato is one of the most significant members of the *Solanaceae* family and is grown extensively in many areas throughout the world. It was first cultivated in Mexico and the Andean region of South America. Nowadays, tomato (*Solanum esculentum*) is the second most important fruit or vegetable crop next to potato (*Solanum tuberosum*), with approximately 189.13 million tons of tomato fruits produced on 5.16 million ha each year (FAOSTAT, 2021).

The tomato fruit has a high nutritional value and may contain approximately 93% to 94% water. It is also an excellent source of minerals, acids, vitamins (A, B, and C), as well as tocopherols, which are beneficial to human health. The tomato fruit contains 3.4% of total sugars, 4.7% of total solids, 15 to 30 mg of ascorbic acid per 100 grams, 7.5 mg of titrable acidity per 100 milliliters, and 20 to 50 mg of lycopene per 100 grams (Chadha, 2012; Naranjo et al., 2016).

It is a self-pollinated annual crop with chromosome number $2n = 2x = 24$ (Peralta et al., 2008). It requires relatively cool, dry climate for high fruits yields and qualities. For growth and development, 20–27 °C is the ideal range. Flooding and waterlogging are not tolerated (Grubben & Denton, 2004). It is also a well-known model species for research on the growth of fruit and the accumulation of phytochemicals. Identifying genotypes with high antioxidant capacities and nutritionally rich phytochemicals is imperative for improving human health. (Raza et al., 2022). To produce tomato crops with the proper agronomical characteristics, it is essential to understand and manage the diversity of tomato genetic resources.

Some intensive production system in greenhouses in Europe uses cultivars with undetermined growth practices and low densities that range from two to three plants per square meter; the stems of the plants are frequently trimmed, resulting in a single stem that reaches a length of more than seven meters; and it is left to harvest fifteen or more bunches per plant in a single crop cycle per year (Chapagain & Wiesman, 2004). Numerous investigations have demonstrated that tomato genotypes with indeterminate growth are the most popular (Meena & Bahadur, 2015; Maciel et al., 2016). According to Sacco et al. (2015), 77.2% of the genotypes taken for cultivation had undetermined growth.

In the intensification of greenhouse tomato production, one of the most important factors is the introduction of new high-yielding varieties and hybrids, which have excellent resistance against diseases and pests and are adaptable to new technologies and unfavorable climatic conditions (Gavrish, 2015). In Romania, tomatoes are grown in greenhouses under conditions of continuous monoculture, which reduces yield efficiency, both quantitatively and qualitatively (Soare et al., 2018). After four years of continuous culture, Bogoescu et al. (2011) showed that the output efficiency dropped to 48%, requiring the application of soil disinfection techniques.

Plant breeding applied on tomato has produced high-yielding varieties, though little attention has been paid to the fruit quality. Kumar et al. (2015) investigated tomato lines for quantitative features such as plant height, fruit yield, fruit weight, total soluble solids, fruit weight loss, and fruit shelf-life in greenhouses as well as plants that were grown in field conditions. They discovered that the plant height ranged from 93.3 to 165 cm in greenhouse circumstances. Fruit weight ranged from 34.4 to 82.0 g, while the total amount of fruit produced per plant ranged from 615 to 1730 g per fruit, and seed lodges were from two to five

Several studies about the genetic structure of tomatoes have devided it into four major groups: processing, fresh market, cherry, and traditional tomatoes or landraces (Williams & Clair, 1993; Robbins et al., 2011; Casals et al., 2018). The first three groups were representative of contemporary tomato cultivars developed by breeders in the 20th century. These tomato varieties were distinguished by their various culinary applications and the introduction of genes from wild species, primarily to increase disease resistance and also to create new cultivars (Blanca et al., 2022). For example, fruits of cherry varieties are tiny and weigh between 2 and 23 g per fruit. The weight of the fruit of normal size varies very much, comprising between 33 and 550 g (Maxim et al., 2023).

In contrast, the term "Landrace" is used to refer to a group of populations or clones of a plant species that are naturally adapted to the local environmental circumstances, as stated in Article 2 of Directive 2008/62/EC. Landraces are also called with many terms like “conservation varieties”, “farmer varieties”, “local varieties”, “primitive varieties”, “local populations”, “peasant varieties” or “traditional varieties” (Conversa et al., 2020; Maxim et al., 2020; Maxim et al., 2023). There are numerous landraces of tomato that might vary in terms of phenotypic and genotypic traits. The number of fruits, first fruiting internode length, fruit weight, fruit yield per plant, number of leaves, and other phenotypic traits are examples of phenotypic characteristics. When different varieties are given the same inputs, such as irrigation and fertilizer, their performances will also differ, and this can be seen clearly from the phenotype of the variety (Marasini & Paudel, 2017).

Traditional cultivars or local landraces selected for a specific area may also be a very suitable genetic pool to increase tomato crop performance. Traditional varieties can be exploited to create new varieties due to their great genetic diversity. Also, they might play a significant part in long-term food security and could help local economies grow (Fullana-Pericàs et al., 2019; Maxim et al., 2020; Scarano et al., 2020). Additional benefits of regional cultivars and landraces include their greater flavor and adaptability for organic farming.

In Romania, the Suceava Bank for Plant Genetic Resources holds 218 landraces and cultivars of tomato. In 2020, there were 77 tomato varieties listed in the official Romanian variety catalogue, of which 64 were appropriate for fresh consumption and 13 for the processing industry (M.A.D.R., 2020).

The study aimed to evaluate the performance of five indeterminate genotypes of tomato to determine their performance under greenhouse conditions at the Vegetable Research and Development Station in Bacau. During the experiment season, some investigations were conducted, such as the number of days until the first flowers appeared, the number of days until the first fruit development, the date of the first harvest, and the outer color of the immature and ripe fruit. In this article, we discuss some phenotypic characteristics such as fruit weight, height, diameter, dry matter content, TSS, water, minerals, and titratable acidity.

To fulfill the rising demand of consumers, breeders need to identify valuable materials featured by tomato plants of the indeterminate variety that produce more, are of superior quality, and are of ideal shape, size, and color. Therefore, there is a need for genetic development and to find potential indeterminate tomato varieties that are suitable for protected production under certain agroclimatic conditions.

MATERIALS AND METHODS

The trial was executed at the Vegetable Research and Development Station in Bacau, during 2022 year, in greenhouse conditions. The greenhouse where the experiment was located is covered with glass, has a height of 3.4 m, and an area of 3000 sq m.

The biological material, represented by five indeterminate local populations of tomato (PL1, PL2, PL3, PL4, and PL5), was collected and conserved from VRDS Bacau. The experiment was established using seedlings obtained into alveolar pallets with 70 alveoli and sowing was realised on February 25, using a textured substrate with a medium fertilization containing microelements. During the growth of the seedlings, four treatments with Previcur and Laser were carried out, a fungicide to prevent the plant falling of tomato, and five foliar fertilization with Green Plant 20: 20: 20.

Manual planting was made when the seedling was well-developed in the second decade of May, when the healthy seedling had the age of 57 days. During the vegetation period, periodic observations on the resistance of pests and diseases and the progress of plant development in protected areas were accomplished. When necessary, manual weeding was done around the plants and at intervals, and for the fight against pests and diseases, treatments with Mospilan were performed. Green Plant were used for fertilizing the plants.

The experiment was divided into four furrows with two rows on each furrow. The distances between the rows are 70 cm, the distance of the passageway between lines of 100 cm. The placement in the plot consisted of five variants; each variant corresponds to two furrows with two rows each. The number of tomato plants for each variant is 48, with 12 plants per row. and a distance of 35 cm between them. The total number of seedlings planted was: 5 variants x 48 plants = 240 plants.

Biological and phenological observations were collected: number of days till appearance of the first flowers, number of days till first fruits development, date of first harvest, the type of inflorescence, as well as the outer colour of the immature and ripe fruit.

The qualitative and quantitative determinations traced the descriptors elaborated by IPGRI (The International Institute for Plants Genetic Resources) with some changes. Some morphological characteristics, such as the diameter and height of the fruits as well as the number of lodges, were noted. The quality of the investigated material cultivated in greenhouse conditions was assessed by determining the total soluble solids (TSS), dry matter (DM), water, minerals, carotene, lycopene, and titratable acidity. The fruits were harvested when physiological stage of maturity was achieved.

Using an electronic caliper that shows accurate and clear data (in inches and mm), the diameter and height of the fruits were measured biometrically, and the weight was estimated using the balance Kern.

The total soluble solids content (TSS) was quantified using a handheld high-precision portable refractometer, and the results were expressed in Brix, according to 932:12 methods (AOAC, 2005). To calculate the dry matter content, freshly collected samples were dried for 24 hours at 103 ± 2°C in a forced air-drying oven (Biobase) to generate a constant mass. Deviations from 100% were indicated by water content. After calcination at 1000°C, the mineral quantity was determined by measurements and reported to be 100% of the fresh weight of the material. Carotene and lycopene pigments were assessed spectrophotometrically.

The titratable acidity of tomato juice was measured following the next method: 50 g of vegetable material was chopped into small pieces and boiled in 250-300 ml of distilled water. Until the material was completely destroyed, it was filled with distilled water, and finally it was allowed to drop below 200 ml. All the extract was filtrated. The titratable acidity of tomato juice was estimated by titrating 10 mL of the tomato juice against a 0.1 N NaOH solution using phenolphthalein as an indicator until the end point was reached. The acid content of the fruit sample was calculated based on the volume of 0.1 N NaOH used for neutralizing the acid content in the sample and multiplying by a correction factor of 0.0067 to estimate titratable acidity as a percentage of malic acid. The titratable acidity was calculated using the following equation: % malic acid = mL NaOH x F x 25 x 2 x 0.0067.

The collected morphological data was analyzed using statistical methods. For statistical analysis, the IBM SPSS Statistics program, version 26.0, was used. Tukey's test was performed in order to estimate the significant difference between the variants means. Differences between groups were considered significant when $p \leq 0.05$. The findings were presented as means with standard deviations.

RESULTS AND DISCUSSIONS

Morphological traits

The results for the investigated local populations with indeterminate genotypes are presented to highlight their performance under greenhouse conditions and to observe the differences and similarities between them. To study earliness, for each variant studied, the following data were observed: date of flowering, date of appearance of the first fruit, date of the first harvest, type of inflorescence, and the exterior color of the immature fruit of the tomato. Referring to the type of inflorescence, each variant had a number of 1-2 ramifications, and the predominant color of the fruit at the immature stage was light green on the entire surface for the numbers PL1, PL2, and PL3, and dark green toward the envelope and light green towards the pistillate tip or PL4 and PL5.

Days to flowering, fruiting and maturity of crop are the important phenological events which determine the productivity of a crop. The days from sowing to fruit ripening ranged from 124 days (PL4) to 131days (PL2). According to the The International Plant Genetic Resources Institute developed descriptors for tomatoes, reaching maturity is defined as when 50% of the plants have at least one ripe fruit. In Table 1, it can be seen that the time required for flowering to begin varied between 69 and 72 days, the time it took for fruit to develop varied between 81 and 86 days, and the beginning of maturation was achieved faster in the PL4 variant, at 124 days.

Table 1. Phenological investigations of tomatoes

Variant	Days till starting flowering	Days till fruit development	Days till harvest
PL1	69.30±0.15c	83.30±0.15c	129.30±0.15c
PL2	72.30±0.15a	86.30±0.15b	131.30±0.15a
PL3	72.30±0.15a	87.30±0.15a	130.30±0.15b
PL4	71.50±0.17b	81.30±0.15d	124.30±0.15e
PL5	67.30±0.15d	80.30±0.15e	126.30±0.15d

Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$.

The predominant color of the fruit at maturity was brick red, which has been observed in four out of five local populations contributing to our collection. The red combination was red-orange, red, bright red, and sometimes red with green streaks. The other color present on the outer surface of the ripe fruits was dark pink, which is associated with the PL4 variant. An excellent fruit shape variability was also observed. In four out of five local populations, the shape the shape of the fruit was round, mostly slightly flattened, while for the PL4 the cordiform shape was representative.

Table 2. Mean values of biometrical measurement of the fruits of tomato

Variant	Height (cm)	Width (cm)	Weight (g/ fruit)	Seed lodges
PL1	6.96±0.32b	9.35±0.51b	365.35±51.83 b	6.00±0.26 b
PL2	6.45±0.22b	8.04±0.23b	253.65±20.19 b	5.40±0.16 b
PL3	6.74±0.18b	8.05±0.23b	249.14±17.93 b	6.00±0.00 b
PL4	10.55±0.16 a	11.82±0.80 a	621.70±59.84a	8.00±0.21a
PL5	6.38±0.12b	8.06±0.18b	236.06±15.85 b	5.40±0.16 b

Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$.

The fruit morphological traits were assessed during the main harvest, by measurements of ten fruits per genotype. The fruit width had average values between 8.04±0.23 cm (PL2) and 11.82±0.8 cm (PL4).

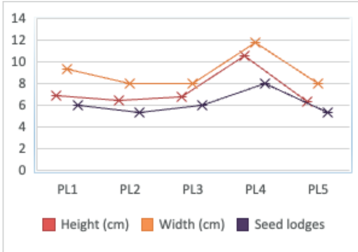

Figure 1. Width, height and seed lodges of tomatoes

According to our investigations, PL1, PL2, PL3, and PL5 can be analyzed together due to its similarly of biometrical measurements (Figure 1). PL1 had the highest values of 6.96 cm height, 9.35 cm width, and 365.35 g fruit weight, while PL5 had the lowest values of height (6.38 cm), PL2 had the minimum width (8.04 cm), and the smallest weight was obtained by PL5 (236.38 g) (Table 2). The variant with the heart shape (PL4) was superior to all other values, and the seed lodges varied between 5 and 8 for all variants.

Biochemical parameters

According to Grierson and Kader (1986), ripe tomatoes present changes in some synthetic chemical elements, such as pigments (beta-carotene and lycopene), aromatic compounds, and some acids (citric and malic), which are primarily in charge of producing the color, flavor, and taste. The primary source of carotenoid intake for humans is the consumption of fruits and vegetables. In this regard, tomatoes are recognized as a primary source of lycopene, followed by β-carotene in tomato fruits, containing 2.62-60.40 mg/100 g fresh weight. The range of lycopene content can vary greatly, depending on numerous factors such as variety, ripening stage, cultivation technology, and geographic location.

The antioxidant activity of lycopene as well as β-carotene along with the abundance of this in tomatoes make this food a rich source of antioxidant activity. Murariu et al. (2021), demonstrate that the lycopene content of eight tomato genotypes cultivated in the plastic tunnel was higher than those cultivated in the field with an average value of 8.44 mg·100 g^{-1}F.W. Literature presents values of lycopene for different varieties of tomato from different regions of the world with mean values between 1.86 to 14.62 mg·100 g^{-1}F.W.

In this research the results for lycopene, β-carotene (expressed as mg 100 g^{-1} of fresh weight - F.W.), and titratable acidity (expressed as % of malic acid) of analyzed tomato varieties are presented in Table 3. The main factors with influence on carotene and lycopene biosynthesis are the genotype and temperature. The pigment content is associated with maturity and also with fruit quality (Brezeanu et al., 2021).

Table 3. Assessment of fruit quality characteristics for tomato landraces tested

Variant	β-carotene mg 100 g^{-1}F.W.	Lycopene mg 100 g^{-1}F.W.	Titratable acidity (% malic acid)
PL1	4.93±0.02e	3.81±0.02e	0.24±0.003d
PL2	7.08±0.005c	5.28±0.04c	0.34±0.009b
PL3	5.15±0,000d	3.96±0.01d	0.38±0.011a
PL4	10.22±0.011a	7.34±0.02a	0.28±0.006c
PL5	7.80±0.000b	5.81±0.04b	0.35±0.006ab

Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$.

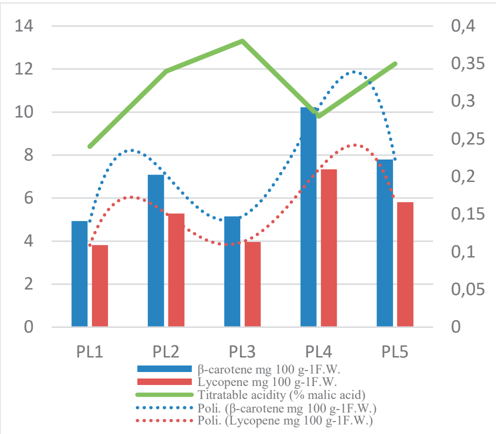

Figure 2. Physiological investigation of tomatoes

The beta-carotene values ranged between 4.93 for PL1 and 10.22 mg 100 g^{-1}F.W. for PL4, which has the highest value (Figure 2). The genotypes studied influenced the lycopene content which values varied within relatively low limits, with values between 3.96±0.01 mg·100 g^{-1}F.W. for PL3 and 7.34±0.02 mg·100 g^{-1}F.W. in the case of PL4, the differences being significant in terms of statistical analysis. Frusciante et al. (2007), analyzed the nutritional value of eighteen tomato genotypes and obtained significant variation among all analyzed genotypes for lycopene and β-carotene content.

Titratable acidity (TA) is an important quality attribute for processing tomatoes, because the higher this value, the easier it is to control microbial deteriorations in processed tomato products such as canned products (Thakur et al., 1996). TA in tomato fruits can be affected by several factors (Lovelli et al., 2017) however the average acidity value of processing tomatoes is 0.35 g 100 g^{-1} FW. In a study conducted in Southern Italy, the mean value of nine tomato genotypes for malic acid was 1255.0 mg kg^{-1}F.W., and varied from 687.0 to 1808 mg kg^{-1}F.W. (Parisi et al., 2022). In our research, the proportion of acid malic was between 0.24 % for PL1 and it raised to 0.35 for PL5.

Table 4. Mean values of physiological characteristics of the tomato landraces

Variant	TSS °Brix	Dry matter %	Water %	MINERALS %
PL1	4.90±0.12a b	4.92±0.007 d	95.08±0.003 b	0.16±0.003 e
PL2	5.27±0.09a	5.52±0.010 b	94.48±0.003 d	0.35±0.003 a
PL3	5.07±0.09a b	5.64±0.003 a	94.36±0.003 e	0.30±0.003 c
PL4	5.33±0.15a	5.22±0.003 c	94.78±0.003 c	0.33±0.003 b
PL5	4.60±0.12b	4.66±0.003 e	95.34±0.003 a	0.28±0.006 d

Values represent the average ± standard error. Within each column, different letters mean significant differences between variants, according to Tukey's test at $p \leq 0.05$.

The results obtained highlight that the highest sugar content, of 5.33 ºBrix, was recorded for PL4, with not significant statstical differences. Regarding genotype factor, the dry matter content varied within low limits, being between 4,66 % and 5.64 %, the values obtained showing, however significant statistical differences. The highest value, of 5.64%, was obtained by PL3, while the lowest value, of 4.66%, was registered by PL5.

In general, the water content of tomato fruits ranged from 92.87% to 96.57% with an average of 94.53% (Athinodorou et al., 2021). Our results confirmed in Table 4 that the water content of tomato fruits is between 94.36% (PL3) and 95.34% (PL5), and the content of minerals is between 0.16% (PL1) and 0.35 % (PL2).

CONCLUSIONS

The present study provides essential information on the morphological and biochemical traits of these tomato local populations. For agriculture, it is crucial to gather and characterize germplasm on a morphological, agronomic, and biochemical level. In this manner, we are able to select the best varieties for different growing environments. It aids breeders in identifying the best parents for novel varieties.

Tomatoes provide a wide range of nutrients, which, along with other components, play a fundamental role in human health. The local populations studied are rich in lycopene and beta-carotene, water, and minerals. The most abundant components in our tomatoes are carotenoids. Also, the rich content of dry matter demonstrates that the fruits of tomatoes present fermity, which facilitates long-term storage.

The local populations with indeterminate growth analyzed in this research showed small differences in the varieties and demonstrated that they could be used as parental genotypes or as pre-breeding material in future variety development for fruit shape, size, color, and flavor desirable for the local market.

Because of the high antioxidant value of tomato genotypes found in current research, it is possible to identify qualitative genotypes that are currently unused and technical practices to produce tomatoes in a system under greenhouse climate conditions.

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STUDY OF THE DISTBUTION OF TOMATO BROWN RUGOSE FRUIT VIRUS (ToBRFV) IN SOUTHERN BULGARIA

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Abstract

The aim of the study is to analyze and investigate viral pathogens on tomatoes and peppers to detect Tomato Brown Rugose Fruit Virus (ToBRFV) in seeds and plant samples. The only hosts of Tomato brown rugose fruit virus are tomato (Solanum lycopersicum L.) and pepper (Capsicum annuum L.). The spread of ToBRFV carries major risks in tomato and pepper cultivation which are important crops grown in Europe. The symptoms resemble those of other viral infections, such as Tomato Mosaic virus, Pepino Mosaic virus and others found in Bulgaria. To achieve the aim of the study and establish ToBRFV over the two-year period, laboratory analyses were performed on more than 28 seed samples of tomates and peppers, imported or in movement within the EC. About 47 plant samples from greenhouses, with the origin of the plants from other countries, were also analyzed. Samples were tested using the ELISA method for five viruses. As a result of this mass screening, the ToBRFV virus was detected on tomato seeds in Bulgaria for the first time. On the recommendation by the phytosanitary authorities, the infected seeds and the tomato plants produced from them were destroyed in order to prevent and limit the future spread of Tomato Brown Rugose Fruit Virus on the territory of Bulgaria.

***Key words**: ToBRFV, ELISA method, spread, destruction, Bulgaria.*

INTRODUCTION

The virus was first detected in 2014-2015 in Israel (EPPO, 2017) and Jordan (Salem et al., 2016), where the incidence in some areas reached 100%. Over the next few years, due to its ability to overcome the resistance of Tm-2/Tm-22 (and Tm-1) resistance genes in tomatoes (Luria et al., 2017), it spread rapidly in production sites. In 2018-2019 it was reported and detected in Italy (Sicily, Panno et al., 2020), Germany (Menzel et al., 2019), Turkey (Fidan et al., 2019), Greece and the Netherlands (EPPO, 2019), where measures were taken to eradicate the infestation. Tomato brown streak virus belongs to the Tobamovirus group and has the characteristic features of this group, such as high stability and infectivity. Mechanical transmission of the virus is highly pronounced, as in tobacco mosaic (TMV), (Kovacevsky, 1970) tomato mosaic (ToMV) (Jones et al., 2014) and other viruses of this group (Broadbent, 1976; Hollings and Huttinga, 1976). The virus is transmitted contactly via contaminated tools, hands, clothing, from plant to plant, infected seeds and planting material, and persists in plant debris and contaminated soil for months to several years (Dombrovsky and Smith, 2017; EPPO, 2020). Tomato brown streak virus (ToBRFV) has no known natural vector (Adams et al., 2016), but possible transmission of tobamoviruses by bumblebees (*Bombus terrestris*) (Levitzky et al., 2019) and birds was reported (Peters et al., 2012). The main hosts of Tomato brown rugose fruit virus are tomatoes (*Solanum lycopersicum* L.) and peppers (*Capsicum annuum* L.) (Luria et al., 2017; Cambrón-Crisantos et al., 2018; Fidan, 2020). ToBRFV symptoms are broad-spectrum and appear on leaves, petioles and fruit. On leaves, symptoms appear first on the young upper part of the plant, such as surface malformation, chlorosis and mosaic with clear chlorotic pale spots. Subsequently, the nervature and leaf petura are deformed, wrinkled and distinct discoloration (browning) is observed, and eventually the plants die. Brown necrotic lesions, similar to ToMV and TMV, are observed on leaf petioles of infected plants, and fruits ripenen unevenly, expressed by the appearance of red, brown and green areas at the same time, symptoms similar to PepMV (Alkowni et al., 2019). With peppers, foliar symptoms include deformation, yellowing and mosaic. Fruits are deformed, with yellow or brown areas or green stripes, plants suffer from slow growth and often die (Luria et al., 2017). The spread of ToBRFV carries great risks when growing tomatoes and peppers which are important crops grown in Bulgaria. On the basis of the EU Decision 2019/1615 a program was established for the monitoring of vegetable crops for quarantine pests and for monitoring and sampling in the production of tomatoes and peppers. Reports of newly discovered and eradicated outbreaks of tomato brown streak virus in neighboring countries (Fidan et al., 2019; EPPO, 2019) and on our territory (EPPO, 2021) stimulated us to investigate the occurrence of this virus in regions with traditions in the production of tomatoes and peppers in Bulgaria.

The aim of the present study is to analyze and investigate tomato and pepper viral pathogens with the aim of identifying tomato fruit brown streak virus (ToBRFV) in seeds and plant samples in regions with a tradition of vegetable production, such as Southern Bulgaria. The analysis of the obtained data will serve to prepare recommendations for the improvement of the currently applied tomato and pepper cultivation technology in greenhouses and outdoors and will affect the increase of producers' incomes.

MATERIALS AND METHODS

A total of 75 samples were analyzed during the serological tests at the Forestry University in Sofia, of which 42 plant, 5 fruit and 28 seed samples originated from Southern Bulgaria. Samples arrived at the laboratory after random visual inspections of plants with symptoms of virus infection or were sent by farmers to assess seed health or problems in greenhouse production from the study areas. 8 samples were analyzed in 2021 and 67 samples in 2022. The leaf and fruit samples with and without symptoms were homogenized 1:20 (w/v) in PBS extraction buffer. Seed samples were soaked at 4°C for 4 h in 1:25 (w/v) extraction buffer, followed by homogenization. In the present study, we used enzyme-linked immunosorbent assay (ELISA) as the most reliable, rapid, highly specific and cost-effective method (Clark and Adams, 1977). DAS-ELISA serological test kits containing antibody (IgG) and alkaline phosphatase conjugate (IgG-AP) for ToBRFV and PepMV detection were from (Loewe, Germany), TMV/ToMV (Sediag SAS, France, Agdia, USA), TSWV and CMV (Loewe, Germany and Bioreba AG, Switzerland) and Loewe controls developed based on existing scientific publications and standards (Luria et al., 2017; Panno et al., 2020; Ling, 2007; De Àvila , 1992; Palukaitis et al., 1992; Albrechtsen, 2006; Blystad et al., 2015; EPPO, 2015). ELISA extraction buffer (pH 7.4); Carbonate buffer (Coating BF, pH 9.6); Conjugate buffer (Conjugate BF, pH 7.4); Diethanol amine buffer (DEA BF, pH 9.8); PBS-T wash buffer (pH 7.4). Antibodies and conjugates at dilutions recommended by the manufacturers were aplied. The reaction results were read using an ELISA reader (spectrophotometer) at a wavelength of λ 405 nm and repeated for correction at λ 495 nm. As a positive reaction, we consider all results showing more than 2 times the values of optical density (OD) readings of the negative control.

RESULTS AND DISCUSSIONS

During the first year of the study, 39 laboratory analyses were performed and on 8 samples and virus infection was detected - in 3 samples from the region of the city of Kardzhali on peppers with TSWV infection and in two samples from the region of the city of Plovdiv from samples of tomatoes with TSWV and pepper plants with CMV infection. After performing the serological laboratory analysis, ToBRFV, TMV, ToMV and PepMV was not detected in 2021 (Table 1).

In 2022, we expanded the survey areas and included those in which there are already reports from the NPPO. Visual inspections in greenhouses revealed signs of virus infection similar to ToBRFV and other viruses, which was confirmed by laboratory diagnostics (Figure 1).

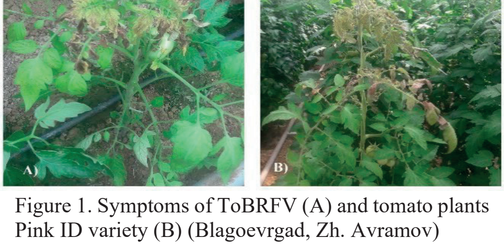

Figure 1. Symptoms of ToBRFV (A) and tomato plants Pink ID variety (B) (Blagoevrgad, Zh. Avramov)

Table 1. Results of laboratory analyzes for ToBRFV, TMV/ToMV, PepMV, TSWV, CMV by the years, host plant, their origin and virus infection detected

Year/No	Culture	Sample	Region	ToBRFV	TMV / ToMV	PepMV	TSWV	CMV	Results +	Virus +
2021/1	Pepper	Fruits	Kardzali	1	2	1	1	1	1	TSWV
2021/2	Pepper	Plant	Plovdiv	1	2	1	1	1	1	CMV
2021/3	Tomato	Plant	Plovdiv	1	2	1				
2021/4	Tomato	Fruits	Plovdiv	1	2	1	1	1	1	TSWV
2021/5	Pepper	Plant	Plovdiv	1	2			1		
2021/6	Tomato	Plant	Vratsa	1	2	1	1			
2021/7	Tomato	Plant	Vratsa	1	2	1	1			
2021/8	Pepper	Seeds	Pazardzhik	1		1		1		
2022/9	Tomato	Seeds	Plovdiv	1	1	1				
2022/10	Tomato	Seeds	Plovdiv	1	1	1				
2022/11	Tomato	Seeds	Plovdiv	1		1				
2022/12	Tomato	Seeds	Plovdiv	1		1		1		
2022/13	Tomato	Seeds	Plovdiv	1		1		1		
2022/14	Tomato	Seeds	Plovdiv	1		1		1		
2022/15	Tomato	Seeds	Plovdiv	1		1				
2022/16	Pepper	Seeds	Plovdiv	1		1		1		
2022/17	Pepper	Seeds	Plovdiv	1		1				
2022/18	Pepper	Seeds	Plovdiv	1		1				
2022/19	Pepper	Seeds	Plovdiv	1		1				
2022/20	Pepper	Seeds	Plovdiv	1		1		1		
2022/21	Pepper	Seeds	Plovdiv	1		1		1		
2022/22	Tomato	Plant	Blagoevgrad	1	2	1	1	1		
2022/23	Tomato	Plant	Blagoevgrad	1	2	1	1	1	1	ToBRFV
2022/24	Tomato	Plant	Blagoevgrad	1	2	1	1	1		
2022/25	Tomato	Plant	Kyustendil	1	2	1	1	1		
2022/26	Tomato	Plant	Kyustendil	1	2	1	1	1		
2022/27	Tomato	Plant	Kyustendil	1		1	1			
2022/28	Tomato	Plant	Kyustendil	1		1	1			
2022/29	Tomato	Plant	Kyustendil	1	2	1	1	1		
2022/30	Tomato	Plant	Kyustendil	1	2	1	1	1		
2022/31	Tomato	Plant	Kyustendil	1	2	1	1			
2022/32	Tomato	Plant	Kyustendil	1	2	1	1			
2022/33	Tomato	Plant	Kyustendil	1		1	1	1		
2022/34	Tomato	Plant	Kyustendil	1		1	1	1		
2022/35	Pepper	Plant	Kyustendil	1		1	1	1		
2022/36	Pepper	Plant	Kyustendil	1		1	1			
2022/37	Pepper	Plant	Kyustendil	1		1	1			
2022/38	Pepper	Plant	Kyustendil	1		1	1			
2022/39	Pepper	Plant	Kyustendil	1		1	1			
2022/40	Pepper	Plant	Kyustendil	1		1	1			
2022/41	Pepper	Plant	Kyustendil	1		1	1			
2022/42	Tomato	Plant	Vratsa	1	2	1	1	1	1	ToBRFV
2022/43	Tomato	Fruits	Vratsa	1	2	1	1	1	1	ToBRFV
2022/44	Pepper	Plant	Vratsa	1	2	1	1	1	1	ToBRFV
2022/45	Tomato	Plant	Smolyan	1	2	1	1	1	1	ToBRFV
2022/46	Tomato	Plant	Vratsa	1	2	1	1	1		
2022/47	Tomato	Plant	Vratsa	1	2	1	1			
2022/48	Tomato	Plant	Vratsa	1	2	1	1			
2022/49	Tomato	Plant	Vratsa	1	2	1	1			
2022/50	Tomato	Plant	Pazardzhik	4		4	1	3	1	ToBRFV
2022/51	Tomato	Plant	Blagoevgrad	1	2	1	1	1	1	ToBRFV
2022/52	Tomato	Fruits	Blagoevgrad	1	2		1	1	1	ToBRFV
2022/53	Tomato	Fruits	Blagoevgrad	1	2	1	1	1	1	TSWV
2022/54	Tomato	Plant	Smolyan	1	2	1	1	1	1	ToBRFV
2022/58	Pepper	Plant	Pazardzhik	1		1	1	1		
2022/59	Tomato	Plant	Smolyan	1				1		
2022/60	Tomato	Plant	Kyustendil	1	2	1	1	1	1	ToMV
2022/61	Tomato	Seeds	Plovdiv	1		1				
2022/62	Tomato	Seeds	Plovdiv	1		1				
2022/63	Tomato	Seeds	Plovdiv	1		1				
2022/64	Tomato	Seeds	Plovdiv	1		1				
2022/65	Tomato	Seeds	Plovdiv	1		1				

2022/66	Tomato	Seeds	Plovdiv	1		1				
2022/67	Tomato	Seeds	Plovdiv	1		1				
2022/68	Tomato	Seeds	Plovdiv	1		1		1		
2022/69	Tomato	Seeds	Plovdiv	1		1		1		
2022/70	Tomato	Seeds	Plovdiv	1		1				
2022/71	Tomato	Seeds	Plovdiv	1		1				
2022/72	Tomato	Seeds	Blagoevgrad	1		1		1	1	ToBRFV
2022/73	Tomato	Plant	Blagoevgrad	1	1	1	1	1		
2022/74	Tomato	Seeds	Plovdiv	1		1				
2022/75	Pepper	Seeds	Pazardzhik	1	1	1		1		
				75	62	72	41	41	15 Positive	

After carrying out 252 laboratory analyses of a total of 67 samples, 10 samples with a positive serological result for ToBRFV were found in 2022 (Table 1). In the region of Blagoevgrad, it was found in 4 samples of tomatoes in plant samples, seeds and fruit (Figure 2). In the Vratsa region, ToBRFV infection was confirmed in 2 samples of plant and fruit tomato and in 1 pepper plant sample. In the region of the city of Smolyan, infection by the virus was detected in 2 plant samples of tomatoes, and in the region of the city of Pazardzhik, a virus infection by ToBRV was detected. In 2 samples of tomato plants, TSWV infection originating from the region of the city of Blagoevgrad and ToMV originating from the region of the city of Kyustendil was detected (Figure 2). The results of laboratory analyses did not prove ToBRFV infection in the regions of the city of Plovdiv, Kardzhali and Kyustendil. The distribution of the results by culture, nature of the samples, surveyed areas, and the number of analyses performed for possible viral infection are shown in Table 2.

Table 2. Positive results of laboratory analyzes for ToBRFV, TMV/ToMV, TSWV, CMV and their origin.

Culture	Sample	No of samples	Region	ToBRFV	TMV / ToMV	PepMV	TSWV	CMV	Results +	Virus +
Pepper	Plants	11	Plovdiv	2	4	1	1	2	1	CMV
			Vratsa	1	2	1	1	1	1	ToBRFV
			Pazardzhik	1		1	1	1		
			Kyustendil	7		7	7			
	Seeds	8	Plovdiv	6		6		3		
			Pazardzhik	2	1	2		2		
	Fruits	1	Kardzali	1	2	1	1	1	1	TSWV
Tomato	Plants	31	Plovdiv	1	2	1				
			Vratsa	7	14	7	7	2	1	ToBRFV
			Pazardzhik	4		4	1	3	1	ToBRFV
			Smolyan	3	4	2	2	3	2	ToBRFV
			Blagoevgrad	5	9	5	5	5	2	ToBRFV
			Kyustendil	11	14	11	11	8	1	ToMV
	Seeds	20	Plovdiv	19	2	19		5		
			Blagoevgrad	1		1		1	1	ToBRFV
	Fruits	4	Plovdiv	1	2	1	1	1	1	TSWV
			Vratsa	1	2	1	1	1	1	ToBRFV
			Blagoevgrad	2	4	1	2	2	2	ToBRFV TSWV
Total amount		75 samples	7 regions	291 laboratory analizes					10 + 1 + 3 + 1 +	ToBRFV CMV TSWV ToMV

The result of one positive sample from the analyzes of 28 samples of tomato and pepper seeds confirmed the claims (Giesbers et al., 2021; EPPO, 2022) that the serological tests for these samples are not suitable for the detection of ToBRFV in seeds. Serological tests for ToBRFV are currently adequate for the identification of the virus in plant samples and fruits, but should be accompanied by others to cover the full nature of the host samples tested. Reports of ToBRFV virus infection in 2022 were confirmed in the region of Smolyan and Pazardzhik and in two new regions of Vratsa and Blagoevgrad in southern Bulgaria, where farmers use imported seeds or seedlings originating from third countries or EU Member states.

Figure 2. Symptoms of infected tomatoes: A) and B) fruits with ToBRFV, Manusa variety; C) plant with ToBRFV, Pink ID variety; D) and E) TSWV on tomato fruits, Pink ID variety (Blagoevrgad region, Zh. Avramov)

CONCLUSIONS

The present study is the first scientific evidence of the spread of ToBRFV in southern Bulgaria. The visual symptoms are close to those of ToMV, TSWV and CMV on the host plants like tomato and pepper. To prove viral infection by ToBRFV in seed samples, the use of molecular identification methods is also necessary. Strictly compliance with phytosanitary measures by all farmers and the recommendations of NPPO to control of ToBRFV is necessary in order to limit the spread of the virus on the territory of Bulgaria.

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THE VARIATION OF CUCUMBER QUALITY DEPENDING ON THE HYBRID AND THE FRUIT HARVESTING INTERVAL

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Abstract

The study evaluated the quality of cucumber production in relation to the hybrids and harvest interval. Five gynoecious cucumber hybrids were cultivated: Madrilène F1 (Seminis), Cantara F1, Kibria F1, Majestosa RZ F1 and Trilogy RZ F1 (Rjik Zwaan). An adequate watering and fertilization regime was ensured. Cucumbers were harvested daily, over a period of 67 days. The highest production of cucumbers was registered with the hybrid Madrilene F1 (Mad), 1034 kg/100 m^2, and the lowest production with the hybrid Trilogy RZ F1 (Tri), 778 kg/100 m^2 in Q1 quality classes (8-12 cm long). The Trilogy RZ F1 (Tri) hybrid had the best share of production in quality class Q1 (97.30%) among all the hybrids under the study conditions (97.10% for Cantara F1 hybrid in Q1; 96.95% for Kibria hybrid in Q1; 96.07% for Majestosa RZ F1 hybrid in Q1, and respectively 91.39% for Madrilene F1 hybrid in Q1). PC1 explained 83.538% of variance, and PC2 explained 7.4489% of variance for the quality class Q1. PC1 explained 59.126% of variance and PC2 explained 19.194% of variance for the quality class Q2.

Key words: *cluster analysis; cucumbers; harvest interval; PCA; quality classes.*

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an annual, herbaceous, fruit-bearing, climbing leguminous plant of the Cucurbitaceae family, originating in Asia, the Southern, tropical areas of the Himalayan Mountains (Qi et al., 2013; Pal et al., 2020).

Cucumber is important for nutrition and health, through the intake of fibers, proteins, carbohydrates, minerals (e.g. calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc) and vitamins (e.g. A, C, K) (Niyi et al., 2019; Mallick, 2022).

Cucumber is grown on a large scale, most often in protected spaces (greenhouses, solariums), as an off-season vegetable, with high yields and economic benefits (Liu et al., 2021; Sallam et al., 2021).

The production potential and the quality of cultivated cucumber genotypes are of interest in breeding programs, and the main objectives are the commercial quality of the fruits, the nutritional quality, and the quality of the fruit aroma (Zhang et al., 2021). The determining factors for the quality of cucumber fruits are considered genetic factors and cultivation factors (technological factors) (Zhang et al., 2021), and a harmonious balance between the two factors categories leads to quality fruits.

Within the cucumber cultivation technologies, various aspects were studied in order to increase the yields, the quality of the production, the optimization of the technologies, in relation to the cultivated genotypes and the growing systems (Dinu et al., 2007; Petre et al., 2016; Biczak et al., 2020; Sallam et al., 2021; Vaudo et al., 2022).

The influence of plant density in relation to the photosynthetic processes of plants, shoot parameters, elements of productivity, production and quality of cucumber production was studied (Ding et la., 2022).

Optimizing the watering regime for cucumbers has been addressed in various studies, for increasing fruit production and increasing water productivity, quantified based on specific parameters and indices (irrigation water productivity, irrigation efficiency, economic productivity, etc.) (Liu et al., 2021).

The influence of some categories of organic fertilizers was evaluated in relation to cucumber productivity, but also to certain chemical properties of the soil in the post-harvest period (Law-Ogbomo and Osaigbovo, 2018). The authors recorded increases in cucumber production and positive changes in some chemical properties of the soil, and in accordance with the cost / benefit ratio, they identified a certain dose of fertilizers, among those tested, as appropriate for the study conditions.

Favorable results of organic fertilizers for cucumbers were quantified based on parameters and physiological indices in plants, simultaneously with the evaluation of the availability and release rate of nutrients from organic fertilizers (Li and Mattson, 2019).

The relationship of some cucumber genotypes (Sultan, Zain) was studied in relation to different fertilizing resources in order to evaluate how the biological material capitalized on the nutritional elements (Al-Bayati, 2020). Based on some physiological parameters and indexes (plant height, number of leaves, leaf surface relative to the plant), and fruit quality parameters (number of fruits on the plant, length of the fruits), the author recorded a different response of the two studied hybrids.

For high yields, different fertilizing substances, organic and mineral, were tested for cucumbers, and based on productivity growth parameters, yield increases were recorded in relation to the fertilized variants of up to 74.6% (Sallam et al., 2021).

The favorable influence of vermicompost, applied in different quantities, was recorded on the basis of physiological indices and plant productivity in cucumbers (Jankauskienė et al., 2022). Some studies have evaluated the performance of cucumber culture in relation to organic and mineral fertilization in growing systems on artificial media, without soil (Adekiya et al., 2022). The authors used growing substrates without soil (coco peat, and rice husk), and provided nutrients through organic and mineral fertilizers (organic fertilizer based on *Tithonia diversifolia*; inorganic fertilizers, as hydroponics fertilizer), they registered considerable increases in production (137%, 198%), and determined the optimal doses for the two categories of fertilizing agents under the study conditions.

The response of cucumber crops to different biostimulant products was analyzed based on physiological indices, growth parameters, starting from seed germination, plant growth, fruiting, production and fruit quality indices (Baratova et al., 2021). The effect of water stress was studied in cucumbers and the possibility of mitigating this water stress through the use of organic and mineral fertilizers (El-Mageed and Semida, 2015).

Some studies evaluated the productivity of cucumbers in relation to different companion crops (Chang et al., 2017), and the authors quantified the influence of this growing system on some attributes of soil quality, microbial activity and the presence of pathogens, associated with the productivity of cucumber plants.

This study evaluated the production of five varieties of cucumbers, in dynamics during the harvest period and by quality classes, in protected culture conditions, modular type solar.

MATERIALS AND METHODS

The study evaluated the production of cucumbers in dynamics, by quality classes under protected culture conditions. The experiment took place in Olari locality, Arad County, Romania.

Five hybrids of cucumbers were cultivated, gynoic hybrids (with female flowers): Madrilène F1 (Seminis), Cantara F1, Kibria F1, Majestosa RZ F1, and Trilogy RZ F1 (Rjik Zwaan). Cucumbers were grown in protected space conditions, modular solar type, with a density of two plants/m^2 (Figure 1).

Watering was provided with an adequate amount of water, in the range of 1-2 L water/plant. Nutrient elements were provided by fertigation, with different fertilizing resources (potassium nitrate, calcium nitrate, magnesium nitrate, urea, complex 27:13.5:0, Ferticare I, II, III).

Harvesting was done daily, between September 16 and November 21, 2020, for a period of 67 days. Cucumber production (kg/100 m^2) was classified by quality categories Q1 (cucumbers 8-12 cm long) and Q2 (cucumbers larger than 12 cm long).

a b c

Figure 1. Aspects from the study period, cucumber culture in protected space; a - aspect after planting cucumber seedlings; b - general aspect from the protected space; c - flowering - fruiting period

In the study, the daily values of cucumber production were grouped by cucumber harvest interval (Chi) of 5 days and recalculated, by hybrids and quality classes.

For reasons of calculation, interpretation of results and graphic representation in the study, certain abbrevi-ations were made and used for the varieties of cucumbers used, the quality classes and the harvesting interval of cucumbers (Table 1).

The ANOVA test, PCA analysis, cluster analysis were used for the statistical analysis and processing of the results. Appropriate statistical parameters were used to confirm the safety of the data (standard error, p, F test, coefficient of variation, cophenetic coefficient). The PAST software (Hammer et al., 2001) and the calculation module from EXCEL were used for the processing and analysis of the experimental data.

Table 1. Abbreviation of terms used in the article

<table>
<tr><th colspan="2">Hybrids</th><th colspan="2">Quality classes</th><th colspan="2" rowspan="2">Hybrid and quality class combination</th><th rowspan="2">Cucumber harvesting intervals (Chi)</th></tr>
<tr><th>Hybrid name</th><th>Abbreviation</th><th>STAS (cucumbers 8 - 12 cm long)</th><th>Under STAS (cucumbers larger than 12 cm)</th></tr>
<tr><td>Cantara F1</td><td>Can</td><td rowspan="5">Q1</td><td rowspan="5">Q2</td><td>Can Q1</td><td>Can Q2</td><td rowspan="5">Chi 1 to Chi 13 for each hybrid and quality class</td></tr>
<tr><td>Kibria</td><td>Kib</td><td>Kib Q1</td><td>Kib Q2</td></tr>
<tr><td>Madrilene F1</td><td>Mad</td><td>Mad Q1</td><td>Mad Q2</td></tr>
<tr><td>Majestosa RZ F1</td><td>Maj</td><td>Maj Q1</td><td>Maj Q2</td></tr>
<tr><td>Trilogy RZ F1</td><td>Tri</td><td>Tri Q1</td><td>Tri Q2</td></tr>
</table>

RESULTS AND DISCUSSIONS

Cucumbers were harvested daily, starting on September 16, until November 21, 2020, for a period of 67 days. Harvested cucumbers were evaluated in relation to dimensional parameters and were grouped into two quality classes: Q1 (fruits 8-12 cm long) and Q2 (fruits larger than 12 cm long) for each variety.

The recorded daily values were grouped by intervals of 5 days and recalculated, in relation to the hybrids studied and the quality classes, and the obtained values are presented in Table 2. The ANOVA test confirmed the statistical reliability of the recorded data series, for each hybrid and harvest interval, as well as the presence of variance in the data set ($F>F_{crit}$, $p<0.001$; $Alpha=0.001$) (Table 3).

Starting from the cucumber production recorded during the study period (67 days), the total production was calculated for each hybrid, and the production for the two quality classes (Q1, Q2), in absolute values and in percentages (Table 4).

Table 2. Cucumber production on hybrids, quality categories and harvest interval

Trial	Kib Q1	Kib Q2	Mad Q1	Mad Q2	Maj Q1	Maj Q2	Tri Q1	Tri Q2	Can Q1	Can Q2
	(kg / 100 m^2)									
Chi 1	42	1	33	2	43	4	33	0	29	1
Chi 2	44	2	39	6	49	5	36	1	34	1
Chi 3	74	3	90	5	92	3	56	2	59	3
Chi 4	65	2	73	3	100	2	76	2	74	2
Chi 5	30	3	43	5	30	0	28	3	34	0
Chi 6	71	3	78	5	74	0	65	0	53	0
Chi 7	67	0	71	6	49	1	46	4	42	0
Chi 8	95	5	119	20	94	12	87	3	94	6
Chi 9	74	1	63	3	53	0	52	0	75	2
Chi 10	60	1	90	14	45	3	47	2	69	2
Chi 11	73	1	74	9	73	2	73	1	80	3
Chi 12	68	2	72	5	64	2	73	2	61	2
Chi 13	94	3	100	6	89	1	85	1	66	1
SE	±5.20	±0.36	±6.79	±1.38	±6.34	±0.88	±5.49	±0.35	±5.53	±0.45

Table 3. ANOVA test, single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	124440.7	9	13826.74	59.93953	3.26E-40	3.379237
Within Groups	27681.38	120	230.6782			
Total	152122.1	129				

Alpha = 0.001

Table 4. Distribution of cucumber production by hybrids and quality classes

Cucumber hybrid	Total production	Production by quality classes			
		Quality class Q1		Quality class Q2	
	(kg/100 m^2)	(kg/100 m^2)	(%)	(kg/100 m^2)	(%)
Kib	884	857	96.95	27	3.05
Mad	1034	945	91.39	89	8.61
Maj	890	855	96.07	35	3.93
Tri	778	757	97.30	21	2.70
Can	793	770	97.10	23	1.90

From the analysis of the obtained data, it was found that the highest production of cucumbers was registered with the hybrid Madrilene F1 (Mad), of 1034 kg/100 m^2, and the lowest production with the hybrid Trilogy RZ F1 (Tri), of 778 kg/100 m^2. From the point of view of placing the fruits in the two quality classes (Q1, Q2), the same positions were kept for the production in Q1, absolute values, respectively the hybrid Madrilene F1 (Mad) with the production of 945 kg/100 m^2 and the hybrid Trilogy RZ F1 (Tri) with the production of 757 kg/100 m^2. As for the weight of the production registered by quality class (expressed in %) of the total production, it was found that the hybrid Trilogy RZ F1 (Tri) had the best weight, of 97.30% in the Q1 quality class, among all the hybrids in study conditions (97.10% for the Cantara F1 hybrid in Q1; 96.95% for the Kibria hybrid in Q1; 96.07% for the Majestosa RZ F1 hybrid in Q1, and respectively 91.39% for the Madrilene F1 hybrid in Q1).

The estimation of the cucumbers production variability, classified according to the registered quality classes, depending on the cucumber harvesting interval (Chi 1 to Chi 13), was made on the basis of the coefficient of variation (CV). For production in the Q1 quality class, high variability was found in the hybrid Majestosa RZ F1, CV_{Maj}= 34.7671, followed by the hybrid Trilogy RZ F1 CV_{Tri}= 34.0092. The lowest value of variability was recorded for the Kibria hybrid, CV_{Kib} = 28.4630, within which the best level was found in terms of the uniformity of cucumbers in the quality class Q1, and the harvest times.

Based on PCA, correlation, the distribution diagrams of cucumber production, in relation to the harvest interval (Chi 1 to Chi 13), and in relation to the studied hybrids (as biplot) were obtained. For the Q1 quality class (Figure 2), the independent positioning of the intervals Chi 1, Chi 2, Chi 5, Chi 7 was found. An intermediate position was observed at Chi 9 and Chi 10. The other harvesting intervals were positioned associated with the genotypes studied. PC1 explained 83.538% of variance, and PC2 explained 7.4489% of variance.

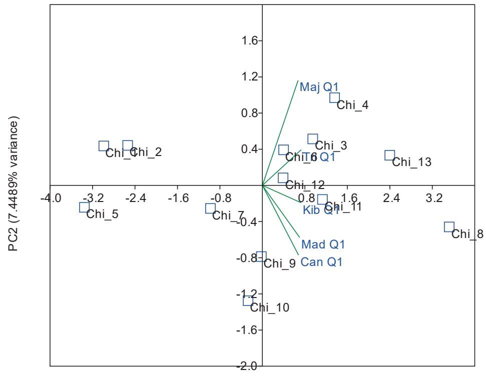

Figure 2. PCA diagram for harvest periods (Chi) for cucumbers and studied hybrids, quality class Q1

For the quality class Q2 (Figure 3), most of the harvest periods were positioned independently, with the exception of Chi 3, Chi 8 and Chi 10; intermediate position were Chi 2 and Chi 11. PC1 explained 59.126% of variance, and PC2 explained 19.194% of variance.

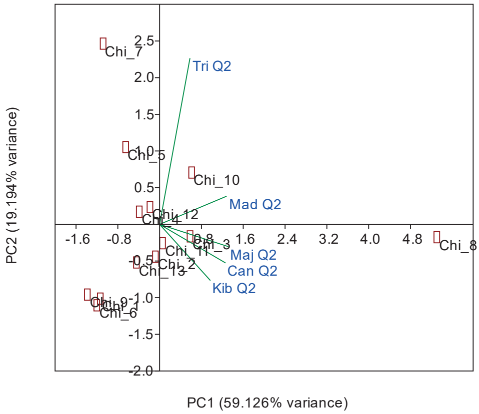

Figure 3. PCA diagram for harvest periods (Chi) for cucumbers and studied hybrids, quality class Q2

The cluster analysis led to the grouping of the variants in relation to the cucumber production per harvest interval and the studied genotypes. In relation to the quality class Q1 (Figure 4), the grouping of the variants based on similarity for the production of cucumbers (intervals Chi 1 to Chi 13), respectively of the studied hybrid, was found, in conditions of statistical safety (Coph.corr = 0.774). A high level of similarity was recorded for the Cantara F1 (Can Q1) and Trilogy RZ F1 (Tri Q1) hybrids.

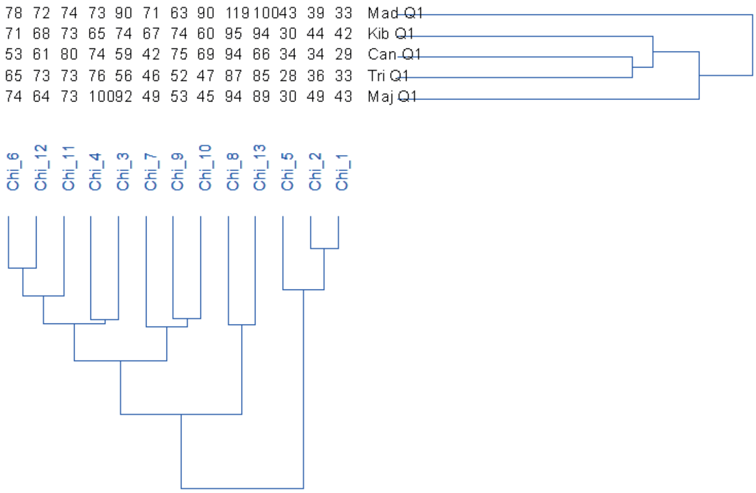

Figure 4. Cluster grouping (two ways) in relation to the production of cucumbers in class Q1

The Madrilene F1 hybrid with the highest values in Chi 8 (110 kg/100 m^2), and Chi 13 (100 kg/100 m^2) was placed in independent position.

Regarding the production of cucumbers by harvesting intervals, a high level of similarity was found for Chi 1 and Chi 2 (low production in Q1, being the first harvests), and Chi 6 and Chi 12, with an average level of the production of harvested cucumbers in Q1. Chi 8 and Chi 13 were associated with the highest production of cucumbers in Q1 (119, respectively 100 kg/100 m^2).

Within the quality class Q2 (Figure 5), the grouping on the basis of similarity of the variants for the production of cucumbers (intervals Chi 1 to Chi 13), respectively of the studied hybrids, was found, in conditions of statistical safety (Coph.corr = 0.949).

A high level of similarity was recorded for the hybrids Kibria (Kib Q2) and Cantara F1 (Can Q2). The Madrilene F1 hybrid was placed in independent position with the highest values in Chi 10 (14 kg/100 m^2) and Chi 8 (20 kg 100 m^2).

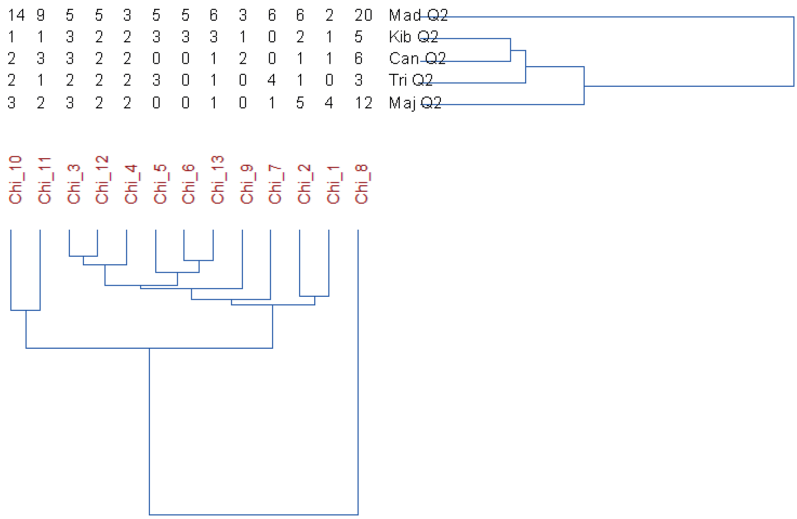

Figure 5. Cluster grouping (two ways) in relation to the production of cucumbers in class Q2

Regarding the production of cucumbers by harvesting intervals, a high level of similarity was found for Chi 3 and Chi 12 (average production of cucumbers harvested in Q2) and Chi 6 and Chi 13, with low production of cucumbers harvested in Q2. Chi 8 was positioned with the highest production (3-20 kg/100 m^2, in relation to the cucumber hybrid).

The five gynoic hybrids of cultivated cucumbers responded differently to the cultivation conditions and the applied technology, response quantified based on the production of cucumbers during the harvest period, and its quality.

The highest total production was recorded for the hybrid Madrilene F1 (Mad), 1034 kg/100 m^2, followed by the hybrid Majestosa RZ F1 (Maj) with a production of 890 kg/100 m^2, and the hybrid Kibria, with a production of 884 kg/100 m^2.

Regarding the production of quality I (Q1), the order of hybrids is maintained, the Madrilene F1 hybrid took first place with a Q1 quality production of 945 kg/100 m^2, followed by the Kibria and Majestosa RZ F1 hybrids with productions of 857 kg/100 m^2, and 855 kg/100 m^2, respectively. In terms of the percentage weight of quality I production from the total production, a high weight was recorded in the hybrids Trilogy RZ F1 (97.30%) and Cantara F1 (97.10%), and the lowest weight was recorded in the case of the hybrid Madrilene F1 (91.39 %).

The differentiated response of two cucumber hybrids to organic fertilization was evaluated in conditions of protected spaces (Al-Bayati,

2020), and the author recorded the differentiated way in which the two tested hybrids capitalized on the nutritional conditions, based on some physiological indices, productivity parameters (number of fruits per plant, yield per plant) and early fruit harvesting, in relation to the nutritional options provided.

The yield variation in cucumbers was analyzed associated with treatments with different biostimulators (Baratova et al., 2021), and the authors reported differential increases in yields (10.28% to 24.27%; 0.9 to 2.7 t/ha) in relation to the applied biostimulator, which shows the receptivity, the capitalization capacity and the differentiated specificity of the plants in relation to the genotype to the elements of technology used.

The increase in cucumber yields was recorded in relation to the addition of organic fertilizers (poultry litter) and minerals (NPK) to the plant growth substrate (Sallam et al., 2021). Through multivariate analyses, the authors of the study identified the experimental variant (combination of substrate, organic and mineral fertilizer) which generated high yield in statistically safe conditions and which can be recommended for similar conditions of study and cultivation of cucumbers.

The quality of plant seedlings and the yield of the cucumber culture were analyzed in relation to different culture substrates, represented by peat with the addition of vermicompost in different percentages (Jankauskienė et al., 2022). The authors of the study communicated the positive effect of vermicompost in relation to the weight of its presence in the substrate, positive effects recorded on the basis of plant height, leaf surface mass of fresh leaves and mass of roots. Associated with these positive effects at the plant level, the yield of cucumbers was 7.4% to 11.1% higher compared to plants grown on peat substrate.

The variation of production residuals and the economic increase in the cucumber culture was analyzed in relation to the irrigation treatments (Liu et al., 2021), and the authors detected the irrigation variants with favorable effects and economic profitability for the study conditions.

Some studies have analyzed the possibility of valorizing organic waste from greenhouse horticultural crops (cucumbers, tomatoes, peppers) in obtaining fodder for animals (Özbilgin and Ince, 2019), and the authors of the study have communicated notable results in this direction.

Such complementary directions for the valorization of organic waste from such horticultural crops can contribute to increasing the degree of valorization of the biological production of these crops, the yields of protected spaces (greenhouses, solariums), but also the energy yields, in the current context of the energy crisis.

The results obtained in the present study highlighted the behavior of the hybrids tested in the study conditions, their differentiated ability to capitalize on the conditions provided by the culture technology.

The variation of production over the harvest interval, and the distribution of production by quality classes calculated over harvest periods, the PCA and CA analysis facilitated the distribution and the clear highlighting of the differences between the analyzed categories, useful aspects in planning the valorization of cucumber production, and adjusting the harvest interval for the foreheads to be included in quality class I (Q1).

CONCLUSIONS

The five gynoic cucumber hybrids studied (Madrilène F1, Cantara F1, Kibria F1, Majestosa RZ F1, Trilogy RZ F1), have differentiated the vegetation conditions ensured by the culture technology, in protected conditions, modular type solar.

During the fruit harvesting period, over an interval of 67 days (September 16 - November 21, 2020), differentiated values of total production and production by quality classes were recorded (Q1, cucumbers of 8-12 cm length; Q2 , cucumbers larger than 12 cm long).

The hybrid Madrilene F1 (Mad) achieved the highest production of cucumbers, 1034 kg/100 m^2, and the lowest production was recorded in the hybrid Trilogy RZ F1 (Tri), 778 kg/100 m^2 in the quality class Q1 (8 -12 cm long).

The Trilogy RZ F1 (Tri) hybrid had the best share of production in the Q1 quality class (97.30%) among all the hybrids grown under the study conditions, followed by the Cantara F1 hybrid (97.10%).

The differentiation of the studied hybrids, in relation to the total production, the production by quality classes (Q1, Q2) and the production per harvest interval of cucumbers (Chi) was made by the multicriteria analysis and confirmed on the basis of the main components (PC1, PC2) and by the analysis clustering.

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PRELIMINARY RESULTS ON THE ACCLIMATIZATION OF A NEW SPECIES OF THE *SOLANACEAE* FAMILY IN ROMANIA

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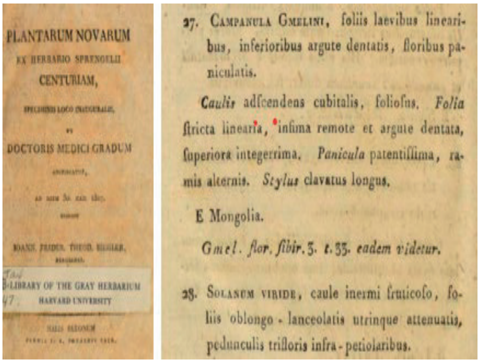
Abstract

The Solanaceae family has been of interest since ancient times, due to the numerous directions of use. Most are decorative species, others are cultivated for their medicinal, insecticidal, and culinary properties. The Solanum melongena species is much appreciated for its high production capacity, but also for the varied shapes, tastes, and textures of the fruits. After the 2000s, in Romania, research on the acclimatization of new species has reached new horizons, with many species being studied. PGRB Buzau has also focused its research on acclimatization and breeding of new species, including Solanum viride, known as poroporo. Although it is native to the Pacific, in particular to the Fiji Islands, the species has shown high adaptability to the climatic conditions of Romania. Plants with a high production capacity, abundant fructification, and increased resistance to the main pathogens specific to the species have been recorded. Research will continue, following phenotypic and biometric determinations, production indices, as well as the chemical composition of the fruits.

Key words*: Solanum viride, poroporo, germplasm collection, genebank, morphotype.*

INTRODUCTION

Around 30,000 of the total of approximately 250,000 flowering plant species in existence are edible (FAO, 1997; Samuels J., 2015). The *Solanaceae* family is composed of more than 100 genera and includes more than 3000 species, spread all over the world. Of these, only a few are widely cultivated and have a significant share in cultivation. The most important species in terms of production are *Solanum lycopersicum* (tomatoes), *Solanum tuberosum* (potatoes), *Solanum capsicum* (peppers), and *Solanum melongena* (aubergines). As a result of climate change and population growth, the range of species grown for fresh or processed consumption has been expanded. Thus, there has been an increase in the biodiversity of cultivated species but also an opening towards new directions of use of the species grown. Following this trend, Romania, through PGRB Buzau, is among the countries that put a special emphasis on improving the vegetable gene pool. Following in the footsteps of the forefathers of Romanian horticultural research, PGRB has dedicated part of its activity to the acclimatization and improvement of new species to the soil and climatic conditions of Romania. Every year, the germplasm collection of the *Solanaceae* family is enriched with dozens of accessions, which are then subjected to acclimatization and breeding works. The natural range of *S. viride* is in the Pacific area, from Fiji to the Pitcairn Islands and the Hawaiian Islands. The exact breeding area of *Solanum viride* is not known, but according to studies, the most accepted area is considered to be Samoa (S. Knapp, 2014).
It is a species with a particular notoriety because of the macabre context of its use. The cannibal communities of Fiji (Seemann, 1862; 1865-73), consumed the fruits and leaves of this species, as the human flesh called *baloka* was difficult to digest, often leading to severe constipation followed by death (A.B. Brewster, 1922).
According to Kew Royal Botanical gardens, the first scientific publication of this species was made in 1807, in the work *Plantarum Novarum ex. Herbario Sprengelii Centuriam* (Figure 1). This book can be found in the Harvard University library.


PLANTARUM NOVARUM
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27. CAMPANULA GMELINI, foliis laevibus linearibus, inferioribus argute dentatis, floribus paniculatis.

Caulis adscendens cubitalis, foliosus. Folia stricta linearia, infima remote et argute dentata, superiora integerrima. Panicula patentissima, ramis alternis. Stylus clavatus longus.

E Mongolia.

Gmel. flor. sibir. 3. t. 35. eadem videtur.

28. SOLANUM VIRIDE, caule inermi fruticoso, foliis oblongo - lanceolatis utrinque attenuatis, pedunculis trifloris infra - petiolaribus.

Figure 1. *Solanum viride* first mention in 1807 (p. 14, pt. 28)

The species was of interest for breeding work, as it has two edible organs: the fruit and the leaves. The fruit in fresh state is bitter, but by thermal preparation this bitterness becomes acceptable, and the leaves are consumed similar to spinach. In terms of biochemical composition, according to Han 1998, it contains solasodine and steroidal alkaloids. Extracts of glycoalkaloids can be used to obtain a potential skin cancer preparation for clinical research (Nada, et al. 2005).

MATERIALS AND METHODS

Beginning with the year 2022, PGRB has taken a new species of *Solanum* under study, namely *Solanum viride*, the *uporo* group.

The crop was established by producing seedlings in alveolar pallets. Peat mixed with sand was used as a substrate. The planting age of the seedlings was about 60 days. Figure 2 shows the planting scheme used by PGRB for the establishment of the culture. Planting distances were 35 cm between plants per row and 70 cm between rows.

The crop was established in open field.

Phenological observations and biometric determinations were carried out during the growing season. These were performed following the UPOV and IBPGRI descriptors adapted from *Solanum melongena*, with particular emphasis on phenotypic stability traits. The colour chart RHS Colour Chart Sixth Edition (2015-2019) was used to determine fruit colour.

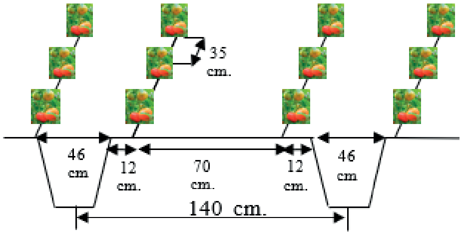

Figure 2. BRGV planting scheme for *S. viride*

RESULTS AND DISCUSSIONS

The general objective of PGRB Buzau is to expand the germplasm collection of the species under study. The *Solanaceae* family is one of the best-represented families in the BRGV Buzău germplasm collection, with more than 10.000 genotypes of tomatoes, peppers, physalis, and aubergines. Besides these well-known species, PGRB has genotypes of *Solanum mammossum*, *Atropa belladonna, Datura stramonium, Petunia spp., Nicotiana tabacum, and Solanum viride.*

Up to now, research on *S. viride* has been mainly aimed at morphological and phenotypic evaluation of the species under Romanian soil and climatic conditions. The process of introduction and acclimatization is the method used for crop improvement. PGRB applies this method by introducing new species in the cultivation area of PGRB Buzau succeeding in time to acclimatize the species of interest for breeding activities. *Solanum viride* was introduced in the process of acclimatization to the soil and climatic conditions of Romania-Buzău. Thus a number of 100 plants were evaluated morphological and phenotypical. The main qualitative characteristics of the plant are presented in Table 1.

Table 1. Main qualitative traits for *Solanum viride* in BRGV Buzau crop

Plant vigour	**medium**
Pubescence	weak
Anthocyanin pigments	absent
Plant growth	subscandent
Leaf color	light green
Flower colour	white
Time of beginning of flowering	medium
Thorns on calyx	absent

The species shows reduced pubescence on the leaves and on the rest of the organs, its presence is very weak. There are no spikes on any vegetative organ. The flowers are small, and white, similar to pepper flowers (Figure 3).

Figure 3. Under microscope aspects of the flower, sepals, stem, and the leaf of *Solanum viride*

Following the qualitative characters, it was observed that so far the species has shown a high degree of adaptability, expressing the specific characteristics of the species in the area of origin, as shown in the images below (Figure 4).

Figure 4. *S. viride* general aspect at BRGV Buzau

The main quantitative characteristics of the species are shown in Table 2. The species showed a well-balanced development, without a large variation of the main measured characters. It ranged in height from 81 cm to 67 cm. The number of shoots was directly proportional to the degree of development of the plants, with plants ranging from 45 cm to 62 cm in diameter in the field. Thus the number of shoots varied from 4 to 8 shoots.

Table 2. Main quantitative traits for *Solanum viride* in BRGV Buzau crop-mean values

Plant height (cm)	**72.8**
Plant canopy (cm)	53.4
No. of branches	6
Leaf blade length (cm)	12.67
Leaf blade width (cm)	8.92
Inflorescence no.	5
Flowers/inflorescence no.	7
Corolla diam. (cm)	3.06
Sepals no.	5-6
Sepals length (cm)	3.14

The plants showed increased resistance to attack by the main pathogens. The resistance of the species under field conditions is remarkable, the plant being in culture also in the second decade of November (Figure 5).

Figure 5. *S. viride* November crop at BRGV Buzau

It has mildly sinuate leaves with reduced pubescence and small dimensions. Light green in colour, without traces of anthocyanins. Leaf size is larger in the basal part of the plant with a length of up to 20.48 cm and decreases towards the apical part to 5.37 cm. Leaf width varies from 11.92 cm to 5.88 cm, in direct correlation with leaf length.
The main qualitative and quantitative characters of the studied fruits are presented in Table 3.
The fruits of the species are distinguished by their features. At the stage of technological ripening, according to the RHS colour charts, they fall into the yellow-green colour group, with code 151 D (Figure 6).

Table 3. Qualitative and quantitative characteristics for *S. viride fruits* - mean values

Length (cm)	**4.75**
Weight (g)	18
Diameter (cm)	5.88
Shape	small pumkin
Fruit/plant no.	50
Pistil scar (mm)	2.68
Fruit apex	indented
Fruit colour	green
Fruit colour intensity	light
Fruit stripes	present
Glossiness at ripness	strong
it ribs	present but weak
Peduncle length (cm)	4.06
Colour of flesh	green
Col. of skin at phys. ripeness	red-orange
Time of physiological ripeness	late
Seed dispersion in fruit	entire fruit
Genetic stability	advanced

Figure 6. Yellowish-green *fruits* of *S. viride*

At the stage of physiological ripening their colour is classified in the orange-red group, with code 33 B (Figure 7). They also have a high gloss at both stages of ripening, and this aspect is preserved for a long time after harvesting

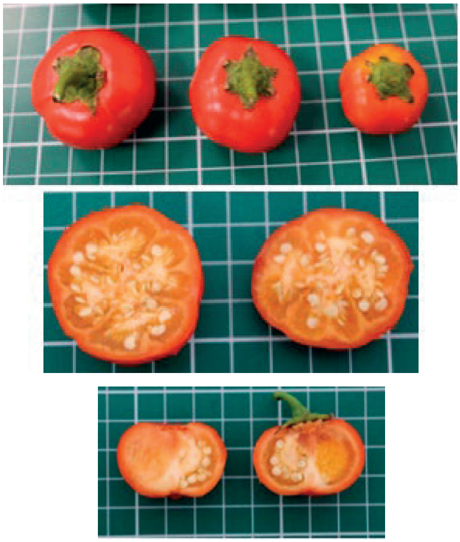

Figure 7. Orange-red fruits of *S. viride*

The number of fruits per plant fluctuates according to the number of inflorescences, the number of fruits in inflorescences and the number of shoots. The number of fruits per plant varied from 35 fruits/plant to 87 fruits/plant, with an average of 50 fruits/plant. On each shoot, 3-5 inflorescences are formed, with the inflorescences in the middle part of the shoot showing the majority of fruits, 6-9 (Figure 8), and the inflorescences in the apical and basal part showing the lowest number of fruits, 2-5.

Figure 8. Inflorescence and fruit aspect of *S. viride*

When the fruit has reached consumption maturity, they can be eaten after thermal preparation. This also contributes to the change in taste, as it has a bitter taste. In their areas of origin they are used in sauces, like tomatoes. When fully ripe, the fruits become soft, fleshy, juicy (Figure 9).

Figure 9 Tomato-like appearance of *S.viride*

They have a high number of seeds in the fruit, that are dispersed throughout the whole fruit. They have a light consistency.

The seeds are surrounded by the gelatinous endocarp of the fruit. They are yellowish in colour, with a reniform shape (Figure 10), a good storage capacity after conditioning and also very good germination potential.

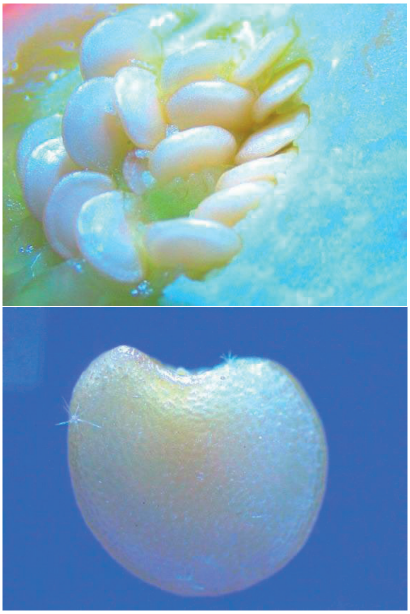

Figure 10. Seed *aspect* of *S. viride* under microscope

CONCLUSSIONS

The introduction of a new species belonging to the *Solanaceae* family in Romania produced promising results in the first years of testing. The plants developed harmoniously and kept the specific characteristics of the species. Following the planting in the open field of 100 seedlings obtained in alveolar pallets, adapting the cultivation technology of *Solanum melongena*, i.e. 35 cm between plants per row and 70 cm between rows, healthy, vigorous and productive plants were obtained. They did not show significant pathogen attacks. It was observed that they prefer well-drained soils, on which waterlogging does not occur. The growth and development of the plants are harmonious, demonstrating a good capacity for acclimatization to the pedoclimatic conditions of our country, especially Buzau, the location of the experiments. They have grown to a height of 67-81 cm, with a direct proportional diameter of the bush of 45-69 cm. The plants have a well-developed stem on which there are a number of 3-5 shoots, each presenting a number of 3-5 inflorescences with 2-9 fruits. They have a long fruiting period, can be harvested in staggered seasons and have a late ripening period. Fruits can be harvested from the second decade of August to the second decade of November. The advantage of this species is that the fruit can be eaten both when green and when fully ripened, their consistency becoming softer, slightly gelatinous on the inside, being suitable for use after thermal preparation such as tomatoes, in various sauces, stews, etc. It has a special commercial appearance conferred by the strong gloss expressed, which persists for many days after harvesting, both at the stage of technological and physiological ripening. This characteristic also ensures a high level of preservation of the fruit after harvesting, with reduced water loss.

The appearance of the plant, conferred by its growth, the green colour of the foliage and the red-orange colour of the fruit also recommend it as a plant with ornamental and decorative value.

Within PGRB Buzău the acclimatization research will continue, and after the completion of this stage, the species will be submitted to breeding works, in order to achieve new genotypes that will contribute to the enrichment of the Romanian vegetable genetic heritage.

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AGRONOMIC PERFORMANCE OF CLIMBING BEAN CULTIVARS IN INTERCROPPING WITH SWEET MAIZE - A PILOT EXPERIMENT FOR PHENOTYPING PROTOCOL DEVELOPMENT

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Abstract

Bean maize intercropping is an old cultivation scheme practiced at small scale by traditional farmers in Romania. Literature highlights the benefits of intercropping and the constrains related extension of this practice on largest areas. Studies on interspecific interactions have paramount importance in view of development of feasible approaches of environmentally friendly cultivation practices and for genetic improvement specific for performance in intercropping. Five climbing bean varieties were evaluated in sole cropping and in intercropping with one type of sweet maize cultivar. Sowing was successively caried out in an interval of 30 days, bean following maize. The performance of bean genotypes in each cropping system was assessed by phenotyping, of different traits. The results show a significant bean genotype × cropping system interaction for flowering and for seed yield. The most competitive bean varieties were taller, more vigorous, and late to maturity. Diversification of cultural systems by implementing bean maize intercropping seems to be an effective strategy in pest management, and further research will provide a basis for incorporating practical pest control schemes into intercropping systems.

Key words*: Phaseolus vulgaris, cropping, phenotyping, yield.*

INTRODUCTION

Progress has often come with social and environmental costs, including water scarcity, soil degradation, ecosystem stress, biodiversity loss, high levels of greenhouse gas emissions, so on. The productive potential of our natural resources has been damaged in many areas around the globe, compromising the future fertility of the planet. Currently, the impact of climate change continually reduces the capacity of these ecosystems to sustain the world's rising food demand. With levels of prosperity as diverse as the cultures, climates, and landscapes, a 'one-size-fits-all' approach does not apply to this dynamic part of the world (FAO, 2021).

Innovative actions are needed to face the emerging threats to food security and nutrition, and to improve resilience.

Diversity of cropping systems as intercropping, crop rotation and crop sequencing may be promising and innovative solutions especially in rain-fed farming areas. A well-designed intercropping system can restore the detrimental effects of intensive farming and monoculture applications, unable to support healthy plant production and contributing cause of plant pests and diseases. Moreover, considering the biochemical profile of species introduced in these cropping systems the natural substances and their secondary metabolites can play an active role as allelopathic or insect repellent to protect the natural sources and the environment by limiting the use of chemicals in weed and pest management and by promoting soil structure and productivity. The system including rotational cropping of cereals with grain legumes and/or cereals with the intercrops of cereals and grain legumes have significant contribution in soil health and N nutrition, as well as breaking the cycles of reducing factors, including insect pests, weeds, and diseases (Nassary et al., 2020). Furthermore, the innovative intercropping system with legume-based rotations promotes farming crop diversification and provide an insurance effect against crop failures and market fluctuations.

More sustainable agricultural systems are necessary to avoid environmental risks with dramatic consequences for inhabitants of rural areas and cities. Such technologies include the use of integrated soil fertility management practices (ISFM) which promote intercropping cereals with legumes as one of its main components (Mucheru-Muna et al., 2010). This practice is an attractive strategy to smallholder farmers for increasing productivity and land labor utilization per unit of area of available land through intensification of land use (Seran and Brintha, 2010). Furthermore, intercropping cereals with legumes retain a huge capacity in replenishing soil mineral nitrogen through the ability to biologically fix atmospheric nitrogen and in reducing interference attributable to weeds and other pests (Giller, 2001). Intercropping of cereals with legumes has emerged as a common cropping system in rain-fed. Intercropping of cereals with legumes improves soil conservation (Anil et al., 1998), favours weed control (Banik et al. 2006), provides better lodging resistance (Anil et al., 1998), yield stability (Lithourgidis et al., 2006), hay curing, and forage preservation (over pure legumes) and may increase crude protein percentage, protein yield, and length of optimum harvest period over grass crops (Qamar et al., 1999). Several factors can affect the growth of the species used in intercropping, including cultivar selection, seeding ratios, and competition between mixture components (Carr et al., 2004).

Brooker et al. (2015) highlighted the challenge of understanding the processes and mechanisms of intercropping could facilitate its manipulation with the purpose to maximize its benefits in terms of yield, soil quality, biodiversity conservation, and farmers' profitability.

To assess the performance of intercropping one pilot experiment was established using bean and maize. The aim was to evaluate the crops growing and development to develop a specific protocol for phenotypic and agronomic investigation of intercropping system.

It is expected that intercropping experiments to contribute to minimizing the soil degradation, nutrient loss and reduced soil and fertility quality, thereby promoting a sustainable soil productivity and microbial community.

Relevant target groups-users, farmers need to be identified, engaged, and involved in the co-design, monitoring, and evaluation possible adoption of this cropping system.

MATERIALS AND METHODS

The experiment was conducted in kind, frame of INCREASE, Intelligent Collections of Food Legumes Genetic Resources for European Agrofood System**,** project as a pilot experiment the major characterisation evaluation intercropping experiment for 2022-2023 planned with three maize varieties and 200 beans varieties. The experiment was also a part of the National project 529 / 2018, aimed to test and develop best cultivation practices for new certified varieties of the unit, as for example in this case, Deliciosul, maize variety.

The location was the experimental field of VRDS Bacău 46.585205 N, 26.950087 E., featured by a climate with 620 mm annual precipitation, and average of multiannual temperature 10.5°C. The soil is well-developed, loamy-sandy textured polished cambic chernozem.

The pilot experiment included five climbing bean varieties in intercropping with one type of sweet maize cultivar.

The bean varieties are the subject of conservation program developed at VRDS Bacau and will be discussed in this work under the codes of CB 1 to CB 5.

The sweet maize used in experiment is a registered variety, *Deliciosul*, highly cultivated all over the county.

'Deliciosul' variety was created at VRDS Bacau. The shape of the cob is cylindrical and the length ranges between 18-22 cm. The number of rows of grains is 13 to 14. The weight of the cob in 300 g at 75% humidity, and the weight of dry cob - 160-200 g, and the diameter 4.2-5.6 cm.

Sowing was manually realised for both crops, and successively caried out in an interval of 30 days, bean following maize.

The performance of bean and maize genotypes was assessed by phenotyping, of different traits. Table 1 includes a part of investigated traits and also traits proposed for the complete protocol of phenotyping. Moreover, during the experimental period monitoring and control of disease and pest species that affects the crops were realized. The observations were accomplished every 10 days. The attack estimation was determined using the following indicators: frequency of attack (F%), intensity of attack (I%), the degree of attack (DA%). The results are useful to control diseases and pests and to manage the number of applied treatments.

The experiment was conducted in three replicates. For each bean variety 20 plants of maize were intercropped. The scheme included one plant of bean for each maize plant. The distance between rows was 70 cm and 20 cm between plants per row.

Previously to sowing fertilization using Linzer Complex NPK and herbicide using Dual Gold were applied.

No inoculation with rhizobia was applied. Two treatments against pest and pathogens using Bactospeine 54% *Bacillus thuringiensis* subsp. Kurstaki and Mospilan 20 SG were applied.

To facilitate the assessment, of influence of intercropping on different traits as flowering, pod sets and maturity, a sole system including same climbing varieties was conducted, using a traditional support system with individual ropes for each bean plant.

RESULTS AND DISCUSSIONS

Intercropping is claimed to be one of the most significant cropping techniques in sustainable agriculture, and much research and many reviews attribute to its utilization several environmental benefits, from promoting land biodiversity to diversifying agricultural outcome. In this sense, intercropping is thought to be a useful means of minimizing the risks of agricultural production in many environments, including those typical of underdeveloped or marginal areas.

This pilot experiment was a preparatory phase for an intercropping experiment developed in three EU locations in Romania, France and Italy, in frame of INCREASE project, during 2022 and 2023. The main purpose was to develop small-scale proof-of-concept studies using maize-bean intercropping, considering the biotic environments as key factors to assess the performance of a system. Maize was considered as a biotic environment for the bean in this agroecosystem. Further we will seek the genomic determinants in bean of the co-adaptation to this intercropping cultivation. The final goals are (1) to predict phenotypic values of individuals and their performance in a given environment for a given trait from their genotype, thereby facilitating the exploration of phenotypically uncharacterized germplasm (2) to develop new cultivation schemes for 'Deliciosul' variety, in order to highlight its cultivation potential.

Our experiment showed is extremely important to select proper varieties in terms of flowering and total vegetation period in close relation with experimental conditions that need to ensure conditions for proper maturation of both crops. The importance of this topic was considered also by Nassary et al., 2020. His approach specified the imperative need of studies to identify the most suitable time of introducing bean in the cropping system. The proposed strategy included moments such as early sowing, sowing mid in the season after a maize crop is well established, and late sowing, in close relation with specificity of environmental conditions.

Table 1. Included traits to phenotypic protocol in order to assess agronomic performance in intercropping

Maize investigations	
days to anthesis (MDA)	days
plant height (MPH)	cm
number of cobs s per plant (MEP)	number
total seed mass per plant (MTSMP)	grams
length and diameter of the cob (CL, CD)	cm
weight of the cob (CW)	g
symptoms of pests and disease (MSPD)	score
Bean investigations	
50% of seeds have emerged (BG)	days
flowering on 50% of the plants (BF)	days
50 % of plants have at least one visible pod (BP)	days
foliage density (BFD)	score
pods placement (BPP)	score
first harvest (maturated pods) (BH)	days
number of pods per plant (BPP)	number
total seed mass per plant (BTSM)	grams
weight of 1000 seeds (MTS)	grams
symptoms of pests and disease (BSPD)	score

Considering maize growing and development during experiment we observed the anthesis occurred in 66 to 78 days, with an average of 66 ± 3 days. During anthesis plants were evaluated in terms of height which variated from 266.33 cm to 290.67 cm.

The shape of the cob was cylindrical, the length registered a small variation between 18 and 22 cm. The weight of the fresh cob varied from 300 g to 375 g.

As a characteristic of investigated variety maize plants developed two cobs per plant.

The bean varieties needed six to eleven days for registering 50% germinated seeds.
Flowering of 50% bean plants was assessed counting the number of days from sowing to half of the plants in the plot had one visible open flower. The variation was large from 27 to 82, with an average value of 54 ± 0.7 days.
Pod set process occurred in 50 to 93 days with an average interval of 60 ± 1.4 days.
The harvest was realized at full maturity when the pods were dry, and the seeds matured. The earliest dry pods were collected after 77 days after sowing and the latest after 134 days. The average value of the interval needed for pods maturation was 96 ± 0.7 days.

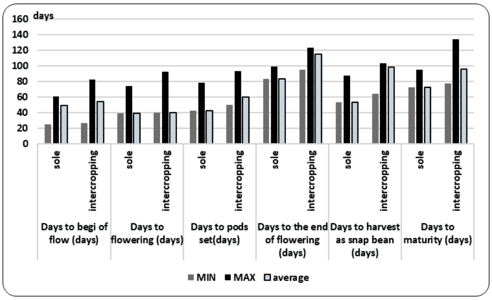

Figure 1. Flowering, pod set, maturity traits of beans in sole and intercropping system

A screening of pest and diseases appearance in bean plots, evidenced the presence of Anthracnose.

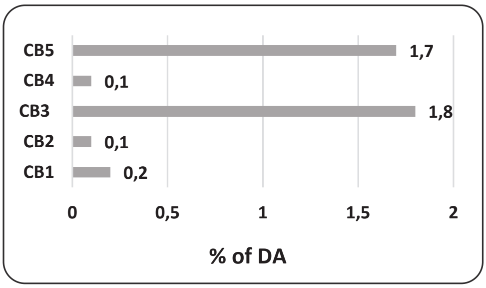

Figure 2. Degree of Anthracnose attack

The symptoms were present on plants starting the middle of June, and attack decreased in July and August. At the harvest moment of bean pods, Anthracnose was observed sporadically on plants of CB3 and CB5 (Figure 2).

Among the pests in the bean plots, the black bean aphid and bean weevil exceeded the economic threshold (ET) and required the application of control treatments.

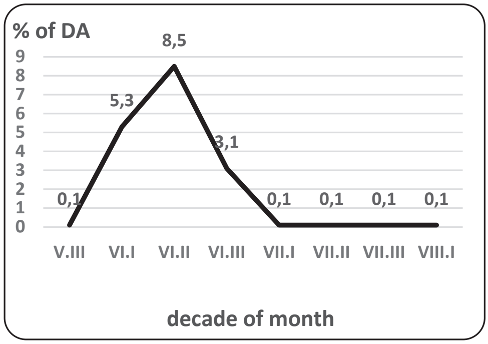

Figure 3. DA% of black bean aphid

The assessment of land utilization advantages of common bean in maize mixtures are recommended to be inserted in the protocol, in respect to specification (Willey, 1979)

This pilot experiment act as support for development of a protocol to be applied in intercropping for characterization and evaluation. The impact is strongly related to bean variability in yields along the years and with the need adapt the intercrop system to certain conditions and influences.

CONCLUSIONS

Our bean maize intercropping pilot experiment provides reasons to include species in cropping in same land, targeting productivity and management of pests and pathogens.
The success of maize bean intercropping system largely depends on the choice of varieties in terms of maturity and capacity to stand (maize), to climb (bean).
Our pilot experiment showed an earliness in case of traits as flowering, pot set up, harvest in case of bean sole system, comparing intercropping. Advantage of maize-bean intercropping system is pronounced in the greater utilization of available resources, benefits in weeds, pests and disease management. More investigations need to be realized to assess the intercropping influence on both crop yield.

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PRELIMINARY STUDY ON TWO LEAFY VEGETABLES GROWN IN DIFFERENT GROWING CONDITIONS IN NFT SYSTEM (*AMARANTHUS VIRIDIS* L., AND *BASELLA RUBRA* L.)

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Abstract

Amaranth and Basella are widely used as leafy vegetables in some countries in Asia and Africa due to their nutritional value. These plants usually cultivate in temperate regions with strong sunlight, so the growth and yield might be restricted under certain conditions, such as cold and low sunlight intensity. The purpose of our study was to assess the specie of amaranth and basella grown in the NFT system at the greenhouse of the *Research Center for Quality Control of Horticultural Produce, Faculty of Horticulture, USAMV. We found that amaranth grows well in natural light conditions, and the yield was doubled compared to LED red light. And basella cultivated in the NFT system gained more productivity and growth compared to cultivation in the pot.*

Key words*: Amaranth, Basella, growing condition, NFT, LED, pot.*

INTRODUCTION

Amaranth is a pseudocereal crop belonging to the family *Amaranthaceae* and the genus *Amaranthus*, comprising 74 species, of which 55 species originated from America, and the other 19 species belong to Eurasia, South Africa, and Australia (Arendt & Zannini, 2013; Waselkov et al., 2018). The species were divided into leafy vegetables and grain types based on utilization (Iftikhar & Khan, 2019).

Amaranth crop is underutilized but it is becoming more and more popular in terms of use as a vegetable, especially in Asia and Africa. It is abundant in protein, minerals, and vitamins (Schafleitner et al., 2022). Amaranth was introduced to Spain in the sixteen centuries, then spread to European nations, and in the nineteen centuries, it extended to Asia and Africa. Since amaranth has nutritional advantages, some European countries, including Germany, Italy, Poland, Czech Republic, Austria, Slovenia, Slovak Republic, Russia, and Romania, have a strong interest in the research and its production (Beatriz Valcárcel-Yamani et al., 2012). Amaranth prefers hot and strong sunlight, especially in tropical and semiarid regions, but cultivated varieties can grow in various regions even under cold climate conditions like in China and Nepal. The ideal pH for the growth of amaranth ranges from 5.5 to 7.5, and with pH 6.4, *Amaranthus tricolor* L., produced more branches, leaves, and a bigger leaf area (Singh and Whitehead, 1992). Ribeiro & Combrink (2006) researched *Amaranthus tricolor* L. using river sand under normal climate conditions and treated with different EC. The result showed that EC 4 mS/cm and EC 8 mS/cm provided the highest yield compared to lower EC treatments. Temperature and light intensity are also significant factors in the growth and yield of Amaranth, for red amaranth, to obtain the maximized yield and betacyanin, it required a temperature between 28-29ºC and light intensity around 1240-1260 $\mu mol.m^{-2} S^{-1}$ (Khandaker, 2009).

Basella rubra L., a perennial vine of the *Basellaceae* family, with a high tolerance to heat. It is also referred to as vine spinach, Malabar spinach, Indian spinach, and Ceylon spinach (Rathee et al., 2010; Roy et al., 2010; Deshmukh S.A; Gaikwad D.K., 2014). Although there is no concrete proof, it has been claimed to be native to Africa and or Asia

(PIER, 2017). Basella is being used as a vegetable in many countries such as India, the Philippines, Indonesia, and some African countries due to the minerals, proteins, fats, carbohydrates, fiber, carotenes, organic acids, and vitamins contains in the leave which makes this plant is beneficial to health. It can be added directly to salads or used as a stand-alone vegetable to make things that are steamed, cooked in oil, or stewed (Singh et al., 2018; Chaurasiya et al., 2021). In hot, humid areas, Basella thrives in full sunlight, in colder climates, it grows slowly and produces limited yields. The ideal temperature for plant growth is 32°C, and when the temperature falls to 26°C, plant development slows. For seed germination, a temperature range of 18-23ºC is ideal. November through February are the short-day months when flowering is triggered. Basella thrives in sandy loam soils with a pH range of 5.5 to 8.0, rich in organic matter (Manju Singh et al., 2016). Cultivation on the NFT system has always been an interesting topic among researchers (Chan et al., 2022; Asmaa et al., 2021), seeing the importance of these plant species due to their valuable nutrients, as well as not much research had been focused on it in NFT system. For this reason, the objective of our study was to examine the growing condition of the NFT system of these species related to its growth and yield component to give some pieces of information for the hydroponic growers and research scientists.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Research Center for Quality Control of Horticultural Produce, Faculty of Horticulture, UASMV, Bucharest, from 15 January to 10 April 2022.

Amaranth seeds were brought from Kbal Koh Vegetable Research Station, Cambodia. The stem and petiole are white, and the leaf shape, ovate, and upper and lower side leaves are green. For basella, the seeds were from Cambodia, a vein plant, that has purple colour on the stem and leaf.

The amaranth and basella seeds were sown with perlite and substrate of the peat Plantobalt type and mixed in the plastic tray (40 x 60 cm) in a ratio of 75% to 25%. The vermiculite-covered row was where the seeds were planted. 7-10 days later, when the seed emerged cotyledon leaves, the young seedlings were moved into Jiffy pots (peat pellets), and manually watered with tap water. After the seedling had 3-4 true leaves or around 4 weeks from the date of seeding, it was moved into NFT. 15 plants of each variety were placed in the NFT system (both natural light and LED red light condition) for Amaranth, and 15 plants of basella I NFT and the pot. Five represented plants of each variety were recorded for amaranth, and four for basella. EC was kept constant at 1.2-14 mS/cm for the first week on the NFT and increased to 1.8-2.2 mS/cm till harvest. pH was 5.8 for the first week and elevated to 6.2 throughout the growing cycle.

For amaranth which is cultivated in the NFT system under both natural light and LED red light conditions. The temperature for natural light was 23-28ºC, and the temperature in nutrient solution was 21-22ºC, with light intensity ranging from 350-500 $\mu mol/m^2s^1$. In LED red light, the room temperature was 20 ± 2ºC, and the nutrient solution was 19-20ºC with 12 hr/12 hr dark/light, and light intensity was 280-300 $\mu mol/m^2s^1$. EC and pH in both conditions were maintained at the same level as mentioned above.

All the data were recorded and done only one time before the flowering stage. For amaranth Leaf width, leaf length, and petiole length of amaranth, three mature leaves were measured for each plant. dry mass was used 1 g of fresh leaves by cutting leaves into small pieces, dried at a constant temperature 105ºC for 24 hours. Chlorophyll content index (ICC) using chlorophyll meter CCM-200 plus, OPTI-SCIENCE. And for basella, all the data were done once, when it reached 75 days from sowing. Regarding the fresh weight, we harvested the whole plant and weighed the stem and leaves together.

The statistical program for analysis was STATISTICA, StatSoft software (version 10) to perform ANOVA analysis at $p \leq 0.05$, 0.01, or 0.001 levels, and Tukey HSD was used to compare the significant difference of each dependent variable at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

There was a significant difference in plant height between the amaranth planted in NFT (natural light) and NFT(LED) conditions at $p \leq 0.05$, (Figure 1). The amaranth cultivated in natural light conditions had higher plant height compared to LED (37.07 and 34.50 cm). However, in both conditions, the number of leaves on the mainstem was not significant difference $p \geq 0.05$ (Figure 1). A significant difference at $p \leq 0.01$, indicated by the number of branches/plant and under the natural light regime produced more branches compared with LED (8.33 and 4.67). At the same time, the total leaves on the branches/plant were also higher under the natural light regime compared with LED light by 60.0 and 49.33 leaves at $p \leq 0.01$. For fresh weight, a highly significant difference at $p \leq 0.001$ was found between the two categories, Amaranth which grew under natural light conditions produced more weight compared with LED light conditions (78.67 and 39.33 g/plant). Our result was obtained similarly to Khandaker (2009), amaranth cultivated under higher temperatures and light intensity produced more plant height and plant biomass. As in our experiment, the temperature and light intensity were higher under natural light conditions, while in LED light were lower and also at the constant point.

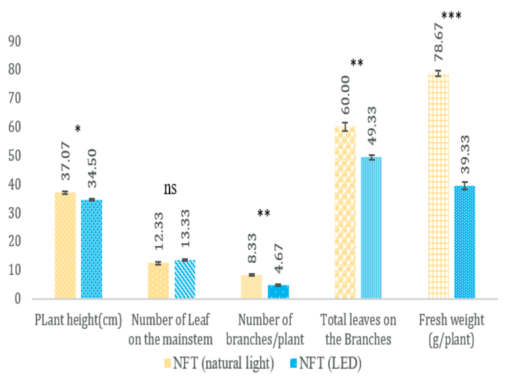

Figure 1. The mean value of plant height, number of leaves on the main stem, number of branches, total leaves number in the branch per plant, and fresh weight (stem and leaves) of Amaranth grown in the NFT system under natural light and LED red light condition. The bar on the graph indicated the standard error, ns-Not significantly different at $p \geq 0.05$. *, **, ***-Significantly different at $p \leq 0.05$, 0.01, or 0.001, respectively (n = 5)

There were no significant differences at $p \geq 0.05$ for leaf width, leaf length, and petiole length (Figure 2). Under both conditions, the leaf width ranged from 9.03-9.70 cm, the leaf length was 13.17-14.08 cm and the petiole was 4.10-4.49 cm. However, stem weight was highly significant at $p \leq 0.001$, the amaranth grown in NFT natural light provided more stem weight compared with LED light (26.0 and 11.67 g/plant). The root length and root volume were also found significant differences at $p \leq 0.01$, under natural light conditions, the root was longer compared with LED light (30.0 and 20.33 cm). This trend was also similar to root volume which plants cultivated under natural light produced more root volume than LED light (22.67 and 8.33 cm^3).

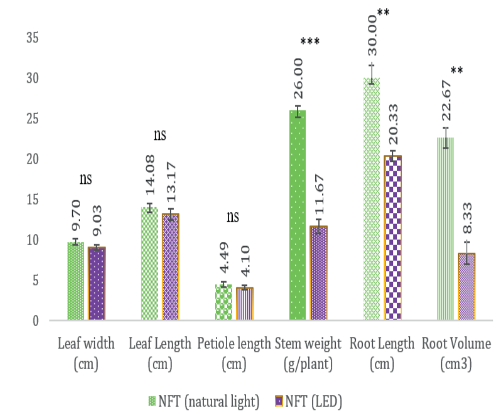

Figure 2. The mean value of leaf width, leaf length, petiole length, stem weight (g/plant), root length, and root volume of Amaranth grown in the NFT system under natural light and LED red light conditions. The bar on the graph indicated the standard error, ns-Not significantly different at $p \geq 0.05$. *, **, ***-Significantly different at $p \leq 0.05$, 0.01, or 0.001, respectively (n = 5)

There was a significant difference in CCI at $p \leq 0.01$, under natural light growing, the CCI contains in Amaranth was higher than LED (12.86 and 8.07) (Figure 3). On contrary, the brix and % of dry matter (leaf) were not different between the plant growing on natural light and LED light. The brix was from 5.87 to 6.07, while the leaf dry matter was 11.56-11.76%. However, % of dry matter of the stem was a significant difference at $p \leq 0.05$, and amaranth grew with LED light was higher in stem dry matter compared to the NFT natural light cultivation.

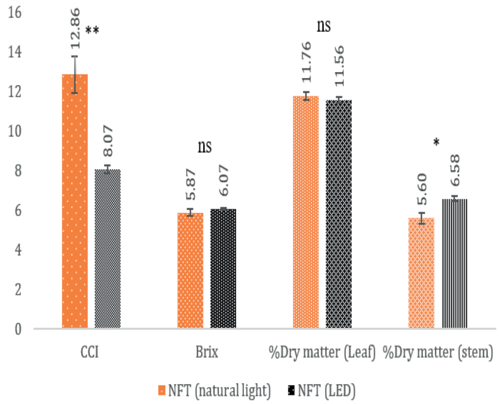

Figure 3. The mean value of CCI, Brix, % dry matter (leaf), and % dry matter (stem) of Amaranth grown in the NFT system under the natural light and LED red light conditions. The bar on the graph indicated the standard error, ns-Not significantly different at p ≥ 0.05. *, **, ***-Significantly different at p ≤ 0.05, or 0.01, respectively (n = 5, and n = 3 for dry matter)

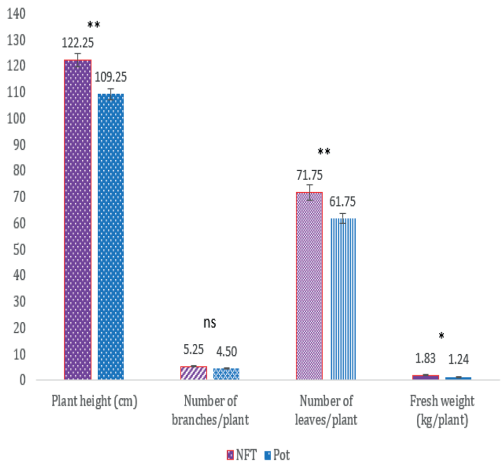

Figure 4. The mean value of plant height, number of branches per plant, number of leaves, and leaf weight of basella grown in the NFT system and in the pot. The bar on the graph indicated the standard error, ns-Not significantly different at p ≥ 0.05. *, **, -Significantly different at p ≤ 0.05, or 0.01, respectively (n = 4)

For basella, there was a significant difference in plant height at $p \leq 0.01$, basella performed well with plant height under the NFT system (122.25 cm), while on the pot (109.25cm) (Figure 4). The number of branches per plant was not significantly different between these two cultures (4.50-5.25). The number of leaves per plant was significantly higher in the NFT system and lower in cultivation in the pot (71.25 and 61.65 leaves/plant) at $p \leq 0.01$. A significant difference at $p \leq 0.05$ presented with the leaf weight, and basella planted in NFT produced more leaves than grown in the pot (1.83 and 1.24 kg/plant).

Figure 5. Amaranth cultivated in the NFT system in both natural light and LED red light conditions

Figure 6. The appearance of Basella plants in culture in the NFT system

a

b

Figure 7. Basella cultivated in the NFT system (a) and the pot (b)

CONCLUSIONS

Based on the results of our studies on the cultivation of amaranth in the NFT system, it was indicated that the plant grown in the NFT system under natural light conditions obtained good growth and yield components including plant height, number of branched, total leaves on the branches, fresh weight and the root development. Amaranth cultivated in LED with low temperature and light intensity, the yield reduced double compared with growing under natural light environment. For the experiment of basella, we found that the best growth was the NFT system, the growth performance and yield were higher than planted in the pot culture.

Further research should be carried out to investigate the growth and yield in the summer season with similar conditions to understand more about the characteristics of two species, as these species prefer warmer weather to reach optimal growth.

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STUDY REGARDING THE INFLUENCE OF DIFFERENT SOWING DATES ON THE PRODUCTION OF SOME *BRASSICA* SPECIES CULTIVATED IN NUTRIENT FILM TECHNIQUE (NFT)

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Abstract

Brassicaceae or the mustard family is one of the popular crops which is cultivated in both open fields and protected culture. It is being used as an oil, condiment, or leafy vegetable type in many countries around the world, and in Asia, it is commonly used as a vegetable due to its nutritional value and health benefits. Usually, growing this type of crop is unpredicted with growth and yield because environmental factors such as temperature, light, humidity, etc might influence during cultivation. The study aimed to compare the growing condition of some Brassica species cultivated in the NFT system. The investigation took place throughout two growing cycles during distinct cultivating times at greenhouse in USAMV. We found the interaction between the variety and growing period in all observed except leaf width and number of leaves per plant.

Key words: *Brassica, species, growing condition, NFT.*

INTRODUCTION

The *Brassica* genus is in the Brassicaceae family. Usually, it is called Cruciferae or the mustard family. It is practically used everywhere in the world, in the form of fresh vegetables, preserved or canned vegetables, oil products, and condiments. Within the *Brassicaceae* family, there are approximately 3709 species, 338 genera, and 25 tribes (Šamec & Salopek-Sondi, 2019; Al-Shehbaz et al., 2006).

The species in this genus were divided into oily, fodder, spice, and vegetable categories, according to Salehi et al. (2021). The six main species include three diploid species, *Brassica nigra* (L.) K. Koch, *Brassica oleracea* L., and *Brassica rapa* L., and three amphidiploid species, *Brassica carinata* A. Braun, *Brassica juncea* (L.) Czern., and *Brassica napus* L., which are important for the world economy. The Brassica family also has a long story regarding plant evolution which current species are related to the wild parents. Dixon (2017) and Arias et al. (2014) reported that the origin of brassicas was in the Horn of Africa and the Arabian Peninsula dating back around 24 million years ago.

For vegetable crops, many factors influence the growth and yield such as temperature, light intensity, CO2, Related Humidity (RH), pH, EC, and fertilization. For instance, Hayat et al. (2009) conducted a study of *Brassica juncea* L. under heat stress at the seedling stage, The plant exposed to both higher temperatures resulted in negative growth, at 40°C, all the growth parameters were decreased such as root length (53.7%), fresh mass (68.3%), and dry matter (65.8%). Siemonsma (1993) said that day length influences flowering, and the bolting happens during a long day without depending on how low the temperature is. When the light intensity was increased from 100 to 600 $\mu mol^{-2}\cdot s^{-1}$ for 16 hours of photoperiod, Jones-Baumgardt et al. (2019) found that the fresh weight of kale, cabbage, and mustard increased by up to 36 percent, 56 percent, and 82 percent. Furthermore, dry weight rose from 65% to 69%, and 145%. When mustard is grown in an open field with solar light intensity below 67 percent, *Brassica juncea*'s growth and yield are reduced (Alam et al., 2018). Dong et al. (2020) reviewed over 100 articles related to the effect of CO_2 on the yield of vegetables, and they found that yield of vegetables increased by

34% when CO_2 elevated to 827 μmol/mol. Roosta (2011) revealed that a pH higher or lower affects plant growth. For instance, pH 8.0 decreased fresh weight, dry weight, and shoot growth. Reduction in shoot growth means the ability of photosynthesis is low, which abrupted the chlorophyll process. While pH 5.0 was best for fresh weight, dry weight, shoot growth, phytochemical yield, and other minerals such as iron, manganese, and zinc. Lower in pH, a soilborne disease easily outbreaks. For instance, clubroot disease usually occurs when the soil pH is lower than 5.7 for cabbage, and mustard crops, while pH from 5.7-6.2 could reduce the occurrence of the disease drastically (Kioke et al., 2003). Samrakoon et al. (2006) found that EC 1.4 dS/m of Albert's solution had a bigger leaf, higher in fresh weight and dry weight. Based on Lee et al. (2012), the optimum EC for red mustard was 2.0-2.5 mS/cm, within this range, the mustard and pak choi increased the yield and vitamin C content. A similar result was also reported by Ding et al. (2018).

Many research efforts have been focused on, especially in the NFT system with some species such as basil, lettuce over different periods, and obtained the best result (Asmaa et al., 2021; Chan et al., 2022; Govoreanu et al., 2022). The objective of our study was to evaluate the growing condition in two different sowing periods on the production of some Brassica crops in the NFT system.

MATERIALS AND METHODS

The study was carried out in the greenhouse under the natural light condition at the Research Centre for Quality Control of Horticultural Produce, Faculty of Horticulture, UASMV, Bucharest, for two growing cycles. The first growing cycle started from 15 January, 2022 to 10 March, 2022 and second growing cycle started from Oct 24, 2022 to Jan 10, 2023 (Table 1).

Mustard varieties were used in the experiment including two varieties of *Brassica juncea* L., V1 is the local variety, seed multiplication and maintained by Kbal Koh Vegetable Research Station, Cambodia, and V2, is from Green Seeds company navigated on the Cambodia's market. These varieties had green colour and a broad leaf, V3 is the specie of *Brassica rapa* var. *parachinensis* or called choy sum from Nam Viet seeds company, had the long petiole and dark green leaves, V4 is the species of *Brassica oleracea* var. *alboglabra* or called Chinese kale (Kai lan 01), from KsSeed company (Figure 1). All the seeds were sown. In the plastic tray (40 x 60 cm) filled with substrate from peat plantobalt type mixed with perlite in the proportion of 75% and 25%. The seeds were placed in the row covered by vermiculite. Seven days after sowing, the young seedlings were transferred into Jiffy pot (peat pellets) and watering manually with tap water.

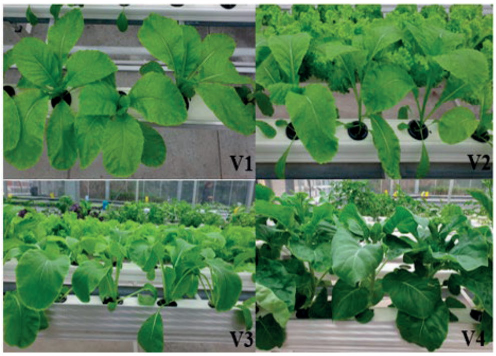

Figure 1. Brassica species grown on Nutrient Film Technique

Table 1. Sowing and harvesting date of the four brassica crops

Variety	Sowing date		Harvesting date	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Brassica juncea L. (V1)	15-Jan-22	24-Oct-22	1-Mar-22	19-Dec-22
Brassica juncea L. (V2)	15-Jan-22	24-Oct-22	1-Mar-22	19-Dec-22
Brassica rapa L.(V3)	15-Jan-22	24-Oct-22	1-Mar-22	19-Dec-22
Brassica oleracea L.(V4)	15-Jan-22	24-Oct-22	19-Mar-22	10-Jan-23
Harvesting day (day)			45-63	56-78

All the seedlings were transferred into NFT when it reached 3-4 true leaves about 21 days from the date of sowing. Each variety were place in NFT with 20 plants and five plants of each variety were observed. EC were maintained at 1.2-14 mS/cm during the first week in the NFT while increased to 1.8-2.2 mS/cm until harvest, and pH was 5.8 during the first week in and elevated to 6.2 during the growing cycle. The average CO_2 was 550-600 ppm, the temperature and humidity were monitored in the greenhouse (Figure 2).

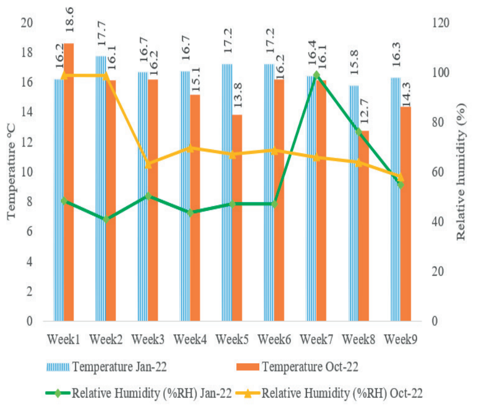

Figure 2. Temperature and relative humidity in the greenhouse in two different growing periods

All the data were recorded at the harvesting stage for five individual plants for each variety including the number of leaves per plant, plant height, leaf width, leaf length, fresh mass using ruler and electronic balance, Brix using reflectometer, Chlorophyll content index using chlorophyll meter CCM-200 plus, OPTI-SCIENCE, nitrate using portable nitrate Greentest ECO, and dry mass using 1 gram of fresh leaves by cutting leaves into fine pieces, dried at constant temperature 105°C for 24 hours. Statistical analysis was used STATISTICA, StatSoft software (version 10) to perform ANOVA analysis at $p \leq 0.05$, 0.01 or 0.001 levels, and Tukey HSD was used to compare the significant difference of each dependent variable at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The average temperature in the first growing cycle started from 16.2°C in week 1 and increased to 17.7°C in week 2, from week 3 and week 4, the temperature remained stable at 16.7°C (Figure 2). However, at week 5 and week 6, the temperature rose to 17.2°C and started to reduce to 16.4; 15.8 and 16.3°C in week 7, week 8, and week 9. For relative humidity, from week 1 to week 6, it ranged from 43-50%, and started to increase in week 7 (99%), then dropped from 76 to 55% at week 8 and week 9.

In the second growing cycle, at the beginning of week 1, the temperature was 18.6°C which was the highest temperature, and dropped to 16.1 and 16.2 in week 2 and week 3. It continued further drop to 15.1 to 13.8°C in week 4 and week 5, then it was back to 16.2 and 16.1°C in week 6 and week 7 but dropped again in week 8 and week 9 (12.7 and 14.3°C). For the relative humidity in the second growing cycle, at the beginning of the experiment, the relative humidity was high, around 99% in week 1 and week 2, then from week 3 to week 8, it ranged from 63-69% and reduced to 58% in week 9.

For brassica species, no significant interaction was found for the leaf width and the number of leaves as it was affected by variety. Plant height, leaf length, chlorophyll content index, fresh mass, dry matter, brix, and nitrate had strong interaction between variety and growing season, while brix was not affected by growing season but within variety itself (Table 2).

Table 2. Interaction of varieties and growing periods over the main effect of four brassica species

	Variety (V)	Growing period (G)	V x G
Plant height	***	**	***
Number of leaves	**	ns	ns
Leaf width	***	ns	ns
Leaf length	***	***	***
Chlorophyll content index	***	***	***
Fresh mass	***	***	***
Dry matter	***	***	***
Brix	***	ns	***
Nitrate	***	***	***

ns-Not significantly different at $p \geq 0.05$. *, **, ***-Significantly different at $p \leq 0.05$, 0.01, or 0.001, respectively.

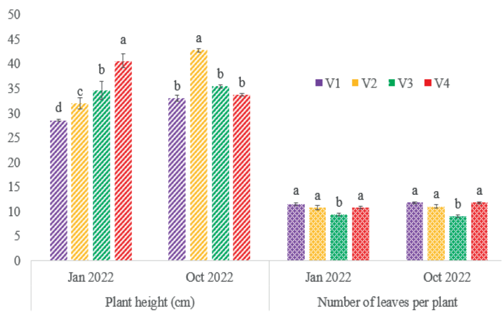

Figure 3. Plant height, number of leaves analysis of four brassica species grown in two different growing cycles in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, $n = 5$

Plant height. There was a strong interaction between the growing periods and the variety over plant height at $p \leq 0.001$ (Table 2). The growing periods influence differently over plant height at $p \leq 0.01$. In the first growing period (Jan 2022), V4 had the highest plant height followed by V3, V2, and V1 (40.6 cm; 34.5 cm; 31.9 cm and 28.5 cm). In the second growing period (Oct 2022), V2 had the highest plant height followed by three other varieties (42.74 cm and 33.0 cm to 35.38 cm) (Figure 3). Overall, growing cycle 2 had 2.31 cm higher than growing cycle 1. Almost no correlation found between the plant height and the fresh mass ($R^2 = 0.0044$) (Figure 9. A).

Number of leaves per plant: There was no interaction between growing periods and the variety at $p \geq 0.05$ (Table 2). The growing periods had no influence on the number of leaves at $p \geq 0.05$. In the first growing cycle, V1 was found higher in the number of leaves (12 leaves), followed by V2, V4, and V3 (11, 11, and 9 leaves) (Figure 3). In the second growing cycle, V1, V2, and V4 had the higher number of leaves (12, 12, and 11 leaves), followed by V3 (9 leaves). There was a slightly low correlation was found between the number of leaves and fresh mass ($R^2 = 0.1032$) (Figure 9. B).

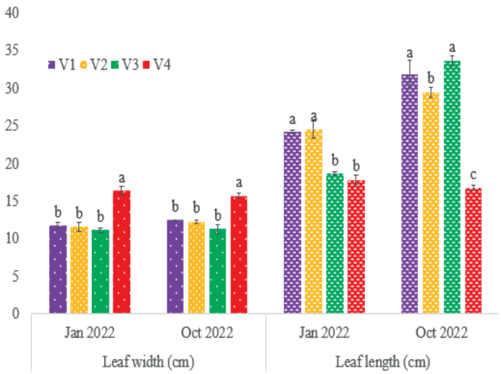

Figure 4. Leaf width, leaf length of four brassica species grown in different two growing cycles in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, n = 5

Leaf width. There was no interaction found between the growing period and the variety over the leaf width at $p \geq 0.05$ (Table 1). Leaf width was unaffected by the growing period at $p \geq 0.05$. In the first growing cycle, V4 had a bigger leaf width (16.37 cm) compared to V1, V2, and V3 (11.75 cm; 11.55 cm and 11.11cm), (Figure 4). In the second growing cycle, V4 remained bigger in leaf width followed by V1, V2, and V3 (15.70, 12.44, 12.26, and 11.25 cm). There was a correlation between the leaf width with the fresh mass ($R^2 = 0.5999$) (Figure 10. A).

Leaf length. Interactions between the growing periods and variety over leaf length were found at $p \leq 0.001$, and leaf length was influenced by growing periods at $p \leq 0.001$. In the first growing cycle, V1 and V2 had similar leaf lengths (24.52 and 24.18 cm) followed by V3 and V4 (18.62 and 17.83 cm) (Figure 4). In the second growing cycle, V3 had a longer leaf length (33.70 cm) than V1, V2, and V4 (31.92; 29.46 and 16.75 cm). In general, Leaf length was affected by the growing cycle 6.67 cm difference between the first and second growing cycles. A negative correlation was presented between the leaf length and the fresh mass ($R^2 = 0.6497$) (Figure 10. B).

Chlorophyll content index (CCI). There was a strong interaction between the growing periods and the variety over the CCI at $p \leq 0.001$. A significant difference at $p \leq 0.001$ was found within the growing periods as well (Table 1). V4 had a higher CCI in both growing cycles (25.59 and 24.49), followed by V3, V2, and V1 (Figure 5). On average 1.66 CCI was found difference between both growing cycles. There was a strong correlation between the CCI with fresh mass ($R^2 = 0.87$) (Figure 11). CCI differed depending on temperatures, consistent with Sharma et al. (2016). Usually, sowing during January gets warmer temperatures compared to October, and from February temperature gradually increasing as the spring began.

Figure 5. Chlorophyll content index of four brassica species grown in two different growing cycles in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, n = 5

Fresh mass: There was also a strong interaction found between growing periods and variety over fresh mass at p≤0.001, and a significant difference at p≤0.001 was observed for growing periods (Table 1). In the first growing cycle, V4 had more fresh mass (120 g/plant), followed by V3, V1 and V2 (77, 58 and 52.6 g/plant). Moreover, in the second growing cycle, V4 still remained more fresh mass (141 g/plant) compared to V1, V3 and V2 (43.17; 42.73 and 37.68 g/plant). The growing periods affected the fresh mass by 10.67 g on average. This result was consistent with Zhou et al. (2022), the yield varies depending on the temperature and the light regimes.

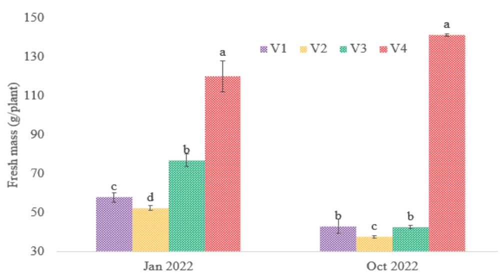

Figure 6. Fresh mass of four brassica species grown in two different growing cycles in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, n = 5

Brix. A strong interaction was presented between the growing periods and variety over brix at $p \leq 0.001$. However, within the growing periods, there was not a significantly different at $p \geq 0.05$. In the first growing cycle, V4 had a higher brix content (5.94), followed by V1, V3, and V2 (5.42, 5.02 and 4.86) (Figure 7).

Figure 7. Brix and dry matter of four brassica species grown in two different growing cycles in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, n = 3

The brix in V4 remained higher (5.90) in the second growing cycle compared with V3, V2, and V1 (5.48, 5.34 and 4.74).

Dry matter. A strong interaction was observed between the growing periods and variety over the dry matter at $p \leq 0.001$, and a significant difference at $p \leq 0.001$ for growing periods. In the first growing cycle, V2 had the higher percentage of dry matter (9.56%), followed by V4, V3, and V1 (8.94, 8.21 and 8.18%), figure 7. V2 and V4 remained higher in the percentage of dry matter (9.53 and 8.72%) in the second growing cycle, compared with V1 and V2 (7.44 and 7.22%). On average, the difference in the dry matter of both growing cycles was 0.50 %. There was a low correlation between the dry matter associated with fresh mass ($R^2 = 0.2929$) (Figure 12).

Nitrate. There was a strong interaction between the growing periods and variety over the nitrate content in fresh produce at $p \leq 0.001$, and a significant difference was also observed for the growing cycle at $p \leq 0.001$. In the first growing cycle, V4 had the highest nitrate content (1834 mg/kg of fresh produce), followed by V1, V3, and V2 (1539, 1432 and 1299 mg/kg). In the second growing cycle, V1 had the highest nitrate content (1166 mg/kg), followed by V4, V3, and V2 (1121, 1106 and 1032 mg/kg). On average, the difference of nitrate between the two growing cycle was 419 mg/kg (Figure 8).

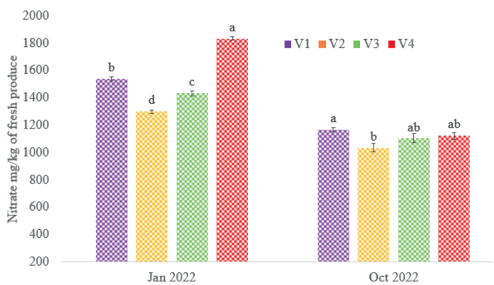

Figure 8: Nitrate of four brassica species grown in two different growing cycles in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, n = 5

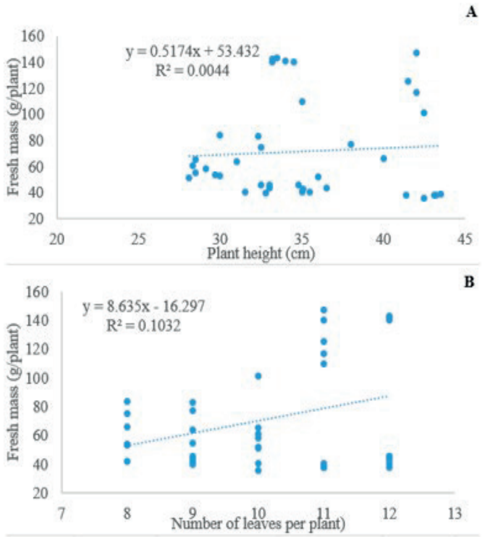

Figure 9. The corelation between plant height with fresh mass (A), and the Number of leaves with fresh mass (B)

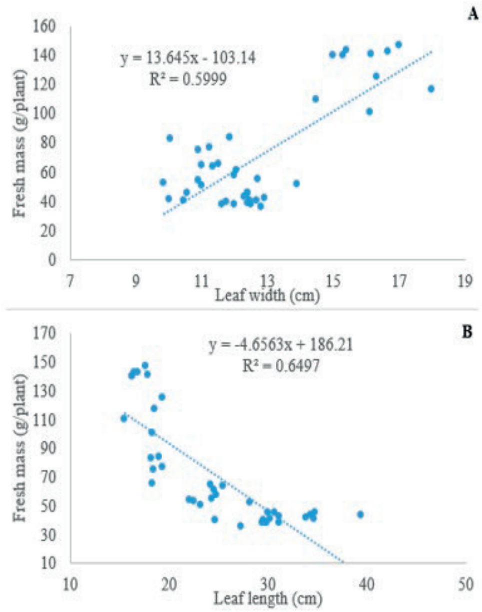

Figure 10. The corelation between Leaf width with fresh mass (A), and the leaf length with fresh mass (B)

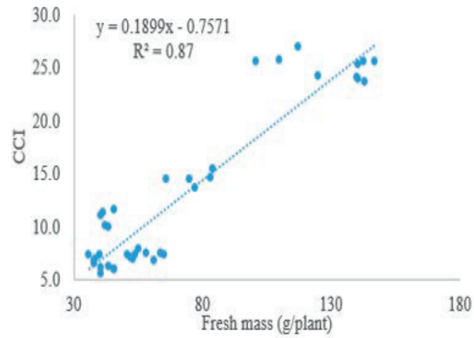

Figure 11. The corelation between CCI with fresh mass

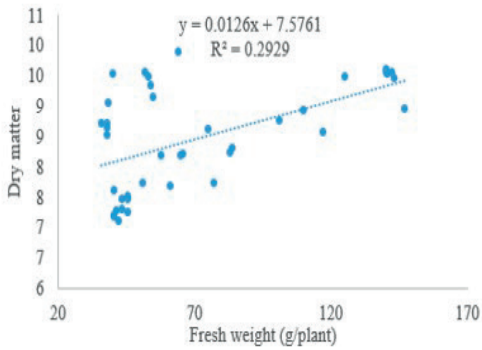

Figure 12. The corelation between dry matter with fresh mass

CONCLUSIONS

Based on the obtained results of our experiments in both growing periods on four brassica species, there was an effect of growing periods with plant height, leaf length, CCI, fresh mass, dry matter, and nitrate.

Overall, in the growing of brassica crops, the growth and yield vary, depending on the characteristic of the species and environmental condition. V4, produced 21.33 g/plant more productivity during Oct 2022 compared with Jan 2022, while the other three cultivars V1, V2, and V3 produced 14.83 to 34.27 g/plant more yield during Jan 2022 than Oct 2022. There were 11-15 days delayed in harvesting when sowed in Oct 2022.

A further observation should be carried out to investigate the specific period of time for the cultivation of the brassica crops, especially during summer and spring.

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EVALUATION OF BIOMASS PRODUCTION OF *STEVIA REBAUDIANA* BERTONI USING CLASSICAL *IN VITRO* CULTURE AND TEMPORARY IMMERSION BIOREACTOR SYSTEM

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Abstract

In vitro cultures can provide a sufficient quantity of high-quality uniform biomass under controlled conditions. The aim of this study was to develop an efficient micropropagation system for biomass production from S. rebaudiana. In vitro shoot proliferation of S. rebaudiana was compared in classical agar-gelled solid culture medium (SM) and the Plantform temporary ımmersion bioreactor system (TIS). Murashige and Skoog 1962 (MS) medium was used in both culture systems and was supplemented with 0, 0.1, 0.2, and 0.3 mg/L 6-Benzyladenine (BA). The maximum biomass production (1396.72 ± 54.03 mg) was recorded using the TIS in the variant with a concentration of 0.2 mg/L BA. The lowest biomass production (229.96 ± 29.33 mg) was obtained on MS solid culture media without BA. It is noteworthy that the water content tended to decrease in TIS compared to SM. In vitro-grown plantlets were screened for possible genetic differences using Start Codon Targeted (SCoT). The PCR amplification products were monomorphic in micropropagated plants and their mother plant, thus proving the genetic fidelity and uniformity of the plants grown in vitro for biomass production.

Key words: *fresh weight, genetic fidelity, solid media, Plantform, Start Codon Targeted Polymorphism*

INTRODUCTION

The development of plant tissue cultures for the production of secondary metabolites has been underway for more than three decades (Yue et al., 2016) because it offers a number of advantages over conventional plant cultivation methods: independence from geographical and seasonal variations, tissue cultured plants are generally free from fungal and bacterial diseases, relatively short growth cycles, mass propagation of a uniform plant material and lack of use of pesticides and herbicides (Debnath et al., 2006).

Therefore, different groups of metabolites have been examined in the biomass of shoots grown in vitro in several plant species such as: biomass of in vitro shoots of *Eryngium species*, shoot cultures of *Ruta graveolens*, tissue cultures of six genotypes of *Deschampsia antarctica*, biomass from in vitro shoots cultures of *Aronia melanocarpa* (Clapa et al., 2022). Likewise, plant tissue culture techniques are increasingly used for the mass propagation of *Stevia rebaudiana* Bertoni and establishing optimal conditions for in vitro cultivation is essential for obtaining high biomass and secondary metabolites production (Miladinova-Georgieva et al., 2023).

S. rebaudiana is a perennial herbaceous plant (family Asteraceae) known as sweet leaf, sweet herbs and honey leaf due to the presence of more than 30 different steviol glycosides found mainly in the leaves (Miladinova-Georgieva et al., 2023). Steviol glycosides are 250-300 times sweeter than sucrose (Peteliuk et al., 2021). Stevia is a valuable source of vitamins, minerals (K, Ca, Na, Mg, Cu, Mn, Fe, Zn), proteins, carbohydrates, dietary fibers, essential amino acids, fatty acids as well as other bioactive compounds such as flavonoids, phenolic compounds, phytosterols, chlorogenic acids and triterpenes (Wölwer-Rieck, 2012; Peteliuk et al., 2021). The benefits of *S. rebaudiana* are numerous, it is used to treat diseases such as diabetes, obesity, tooth decay, hypoglycemia and hypertension (Faramayuda et al., 2022).

In vitro propagation of *S. rebaudiana* has been studied by many researchers to develop different micropropagation protocols in semisolid media gellified with agar (Sivaram & Mukundan, 2003; Tadhani et al., 2006; Kalpana et al., 2009; Sairkar et al., 2009; Pratibha et al., 2010; Ali et al., 2010; Singh & Dwivedi, 2014; Razak et al., 2014; Soliman et al., 2014; Kaplan et al., 2019; Yesmin, 2019) or in temporary immersion systems (TIS) (Norazlina et al., 2012; Sacco et al., 2013; Ramírez-Mosqueda et al., 2016; Vives et al., 2017; Rosales et al., 2018; Bayraktar, 2019; Melviana et al., 2019; Saptari et al., 2022). Furthermore, several plant tissue culture techniques have been developed for mass propagation, biomass and secondary metabolite production of *S. rebaudiana,* such as: callus culture, suspension cultures, adventitious root culture (Kazmi et al., 2019).

Hence, plant tissue culture techniques can provide the production of plantlets which are genetically uniform and have homogeneous secondary metabolite content within a short time under controlled physical conditions (Bayraktar et al., 2016). Currently, molecular markers are valuable biological tools for analysing the genetic fidelity of micropropagated plants (Pandey et al., 2021). The aim of this study was to develop an efficient micropropagation system for biomass production from *S. rebaudiana* and to evaluate the clonal fidelity of in vitro-grown shoots through DNA-based molecular markers. In vitro biomass production of Stevia was compared in classical agar-gelled solid culture medium (SM) and the Plantform temporary immersion bioreactor system (TIS). The genetic stability of in vitro-grown plantlets was assessed by Start Codon Targeted (SCoT) molecular markers.

MATERIALS AND METHODS

Biomass production in solid and TIS culture systems

The explants used for this experiment were excised from in vitro cultures of *S. rebaudiana* (4 weeks old) which had been cultured on Murashige and Skoog medium (MS) Murashige & Skoog, 1962) with 3% (w/v) sugar, 5 g/L Plant agar, pH 5.8, without growth regulators.

For the production of biomass, two *in vitro* culture systems were used: solid (SM) and liquid (TIS). For solid media, 370 ml jars were used as culture vessels, which were 7 cm in diameter and 12.7 cm high, with screw caps, containing 50 ml media/jar. TIS culture systems used Plantform bioreactors (Welander et al., 2014), with a volume of 400 ml media/ bioreactor. The immersion time was one minute at four-hour intervals and the aeration time was four minutes at one-hour intervals. The basal culture medium used for both types of culture systems was MS with four concentrations of BA, 0, 0.1, 0.2, and 0.3 mg/L, respectively. The SM was solidified with 5 g/L Plant Agar. The liquid culture media and those jellified with agar were sterilized for 20 minutes in the autoclave at 121°C and 0.11 MPa. All the components of the culture media were purchased from Duchefa (Biochemie B.V, Netherlands), and they were added prior to sterilization, as well, the pH was adjusted to 5.8.

The culture vessels with solid media were inoculated with 5 shoot fragments, whereas the bioreactors were inoculated with 20 shoot fragments, each of them 1.5-2 cm in length.

The in vitro cultures were incubated in the growth room at 16-hour photoperiod with 32.4 μmol $m^{-2}s^{-1}$ light intensity (Philips Core Pro LED tube 1200 mm 16 W 865 CG, 1600 lm Cool Daylight) at 23 ± 3°C and 50 ± 2% humidity.

Genetic fidelity analysis using SCoT markers

The genetic fidelity between the in vitro obtained stevia shoots and their mother plant was assessed using SCoT markers. The biological material was represented by leaves of the in vitro proliferated shoots from the two different culture systems and from their mother plant as control. The harvested leaves were dried, ground into a fine powder (Tissue Lyser II, Qiagen, Germany) and kept at 4°C until the genetic analyses were carried out.

Total genomic DNA isolation. DNA was isolated from four randomly selected shoots for each culture media variant and each in vitro culture system. The dry material represented by 0.2 g of leaf powder was processed using a protocol based on the CTAB (cetyltrimethylammonium bromide) method as published by (Lodhi et al., 1994) and improved by (Pop et al., 2003) and (Bodea et al., 2016). The DNA concentration and purity were determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to performing the PCR (polymerase chain reaction) amplifications, all the DNA samples were diluted to 50 ng/ µL using nuclease-free water.

SCoT analysis. For the SCoT analysis, the PCR amplifications were carried out using the protocol described by (Collard & Mackill, 2009). The reaction mixture (a total volume of 15 µL) consisted of 50 ng/ µL of gDNA, distilled H_2O for the PCR reactions, 5X GoTaq Flexi Green buffer (Promega, Madison, WA, USA), 1.5 mM $MgCl_2$ (Promega, Madison, WA, USA), 0.2 mM of dNTP mix (Promega, Madison, WA, USA), 1 µM SCoT primer (GeneriBiotech, Hradec Králové, Czechia), and 1U of GoTaq polymerase (Promega, Madison, WA, USA). The PCR temperature cycling conditions were: (a) 1 cycle of 5 min at 94°C for initial denaturation, (b) 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 2 min, and (c) the final elongation step of 7 min at 72°C. The SCoT primers used in this study are shown in Table 1.

Table 1. The list of SCoT primers used

Primer name	The 3'-5'nucleotide sequence of the primer
SCoT 9	CAACAATGGCTACCAGCA
SCoT 10	CAACAATGGCTACCAGCC
SCoT 12	ACGACATGGCGACCAACG
SCoT 15	ACGACATGGCGACCGCGA
SCoT 16	ACCATGGCTACCACCGAC
SCoT 18	ACCATGGCTACCACCGCC
SCoT 19	ACCATGGCTACCACCGGC
SCoT 21	ACGACATGGCGACCCACA
SCoT 22	AACCATGGCTACCACCAC
SCoT 23	CACCATGGCTACCACCAG

The PCR amplifications were repeated twice for each SCoT primer to ensure the reproducibility of the results. Separation of the PCR amplified products was performed by electrophoresis on 1.4% agarose gels (Promega, Madison, WA, USA) stained with RedSafeTM Nucleic Acid staining solution (iNtRON Biotech, Seoul, South Korea) in 1X TBE (Tris Borate-EDTA buffer), at 110 V and 136 mA for 2.5-3 h. The electrophoretic profiles were visualized under UV (ultraviolet in UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Upland, CA, USA).

Data Collection and Statistical Analysis

Each *in vitro* experiment was repeated three times, more exactly three jars per repetition and three bioreactors per repetition. Stevia shoots grown *in vitro* on solid and liquid media were collected for analysis after 4 weeks of culture. Data analysed refer to the average length of shoots (SL), the fresh weight (FW), dry weight (DW) and water content (WC). FW was obtained by weighing the shoots immediately after the material was removed from the in vitro culture medium. To obtain DW the material was dried for three days at 45°C and weighed again. WC percentage was calculated using the formula (Mazurek et al., 2021):

WC (%) = [(Fresh Weight-Dry Weight)/Fresh Weight] *100

ANOVA was performed followed by Tukey's HSD test ($P \leq 0.05$) to determine the statistically significant differences between the means. Values shown (in text and figures) are means ± SE (standard error).

RESULTS AND DISCUSSION

For biomass production, two different culture systems (solid-gellified with agar and TIS) were compared in terms of growth parameters in *S. rebaudiana*.

Regarding shoot lengths (Figures 1 and 2), they recorded maximum values in TIS, on MS medium supplemented with 0.2 mg/L BA (11.91 ±0.54 cm), while the shortest shoots were obtained in MS solid media supplemented with 0.3 mg/L BA (4.40 ± 0.19 cm).

Previous studies used BA (BAP) in various concentrations for shoot induction in the MS solid media or TIS (Kalpana et al., 2009; Ali et al., 2010; Norazlina et al., 2012; Hassanen & Khalil, 2013).

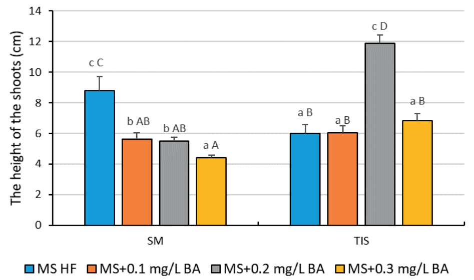


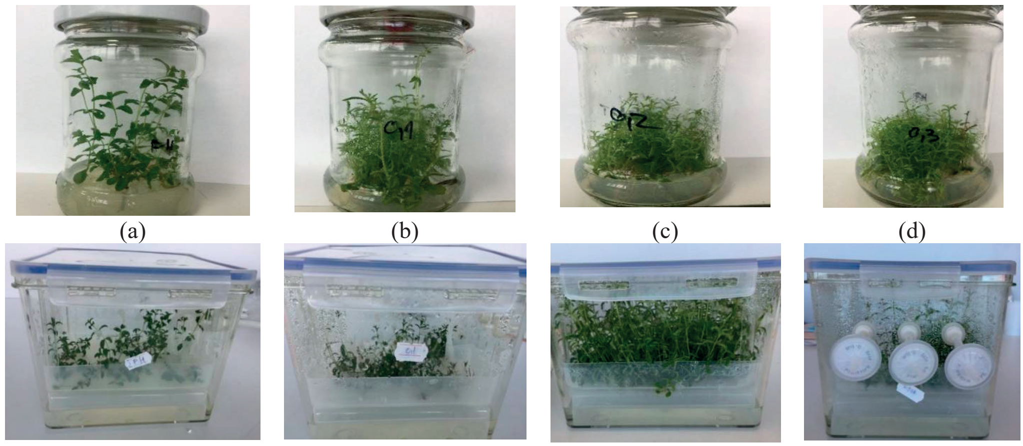
Figure 1. Shoots length (cm) of *S. rebaudiana* cultivated on MS medium supplemented with 0, 0.1, 0.2 and 0.3 mg/L BA, in two different culture systems: solid culture medium (SM) gelled with 5g/L agar, and liquid culture medium through temporary immersion (TIS) system in Plantform bioreactor. Lowercase letters above the bars indicate significant differences between the means of the shoots length obtained on the same culture system; capital letters above the bars represent significant differences between the means of the shoots length obtained on the both culture systems according to Tukey's HSD test ($P \leq 0.05$)

On a culture medium similar to ours but solidified with 0.8% (w/v) agar and supplemented with higher concentrations of BAP (0.5, 1.0, 1.5 and 2 mg/L) Hassanen & Khalil (2013) reported that shoot length of stevia decreased from 3.84 cm to 0.74 cm with increasing BA concentration.

In general, these studies show that BA, in low concentrations, is an adequate cytokinin for *in vitro* multiplication in *S. rebaudiana*, a fact supported by our results.

Fresh weight is a very important parameter in biomass production for obtaining bioactive compounds. Growth measurements in terms of fresh weight were taken at the end of the four-week culture cycle. After weighing the freshly removed inocula from the cultured media it was noticed that all the concentrations of BA increased fresh weight in both culture systems, but FW increase of the cultures differed between bioreactor and agar medium. The fresh weight proved to be statistically significantly higher on 0.2 mg/L BA in TIS, 1396.72±54.03 mg/inoculum, respectively (Figure 3a).

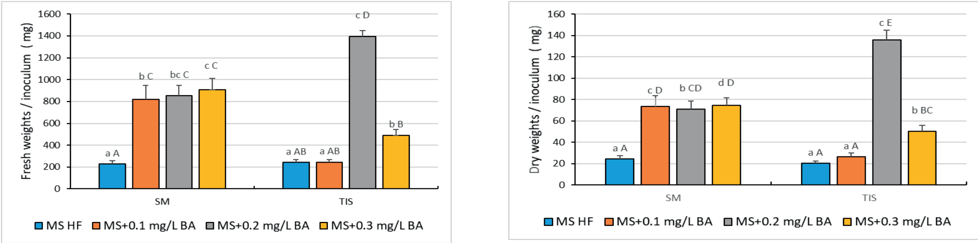
However, in TIS, higher concentration of BA (0.3 m/L) resulted in a decrease in fresh weight (491.36 ± 52.05 mg/inoculum).



(a) (b) (c) (d)

(e) (f) (g) (h)

Figure 2. *In vitro* cultures of *S. rebaudiana*, cultured on MS medium supplemented with different concentrations of BA, in two culture systems: solid culture medium gelled with 5 g/L agar (SM) without BA (a), SM supplemented with 0.1 mg/L BA (b), SM supplemented with 0.2 mg/L BA (c), SM supplemented with 0.3 mg/L BA (d); liquid culture medium through TIS in Plantform bioreactor without BA (e), TIS supplemented with 0.1 mg/L BA (f), TIS supplemented with 0.2 mg/L BA (g), TIS supplemented with 0.3 mg/L BA (h)



(a) (b)

Figure 3. Fresh weights /inoculum (mg) (a) and dry weights /inoculum (mg) (b) of *S. rebaudiana* cultured on MS medium supplemented with 0, 0.1, 0.2 and 0.3 mg/L BA, in two different culture systems: solid culture medium (SM) gelled with 5g/L agar, and liquid culture medium through TIS system. Lowercase letters above the bars indicate significant differences between the means of the fresh weights obtained on the same culture system; capital letters above the bars represent significant differences between the means of the fresh weights obtained on the both culture systems according to Tukey's HSD test (P≤0.05)

The maximum FW production on the solid medium was achieved at a concentration of 0.3 mg/L BA, 909.31±101.37 mg/inoculum, respectively. In both culture systems the lowest FW was obtained on culture medium without BA, respectively 229.96 ± 29.34 mg/inoculum on SM and 244.32 ± 22.71 mg/inoculum on TIS (the difference is not statistically significant). The dry weight ranged from 20.46 ± 2.06 to 135.69 ± 9.39 mg/inoculum for TIS and 24.41 ± 2.88 to 74.42 ± 7.45 mg/inoculum for agar medium (Figure 3b). After drying the leaves the total water content was determined. The determinations revealed an increasing tendency in water content with the increasing of BA concentrations for both culture systems (Figure 4).

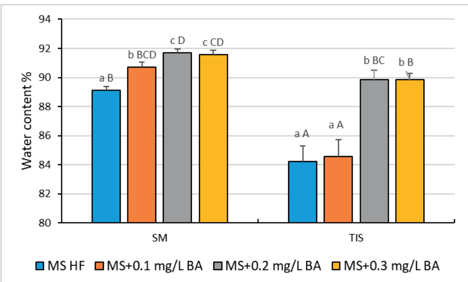


Figure 4. Water content (%) of *S. rebaudiana* cultivated on MS medium supplemented with 0, 0.1, 0.2 and 0.3 mg/L BA, in two different culture systems: solid culture medium (SM) gelled with 5g/L agar, and liquid culture medium through temporary immersion (TIS) system in Plantform bioreactor. Lowercase letters above the bars indicate significant differences between the means of the shoot length obtained on the same culture system; capital letters above the bars represent significant differences between the means of the shoot length obtained on the both culture systems according to Tukey's HSD test (P≤0.05)

In this case the WC of the proliferated shoots in the in SM showed an increase from 89.10 ± 0.28% for MS without BA to 91.69 ± 0.24 % on media supplemented with 0.2 mg/L BA. The proliferated shoots in TIS culture system showed an increase of WC from 84.21 ± 1.07% (MS without BA) to 89.86 ± 0.61 % on media supplemented with 0.2 mg/L BA. Our results show that the shoots obtained in TIS had a higher WC compared to those obtained in SM. It should be noted that WC is higher in TIS compared to SM at all concentrations of BA. Previous studies have also shown significant differences in fresh and dry weight or water content in *S. rebaudiana* or other species grown in TIS and agar medium. Thus, Vives et al (2017) reported a 6.6 times higher FW production in temporary immersion bioreactor (BIT®) compared to solidified medium with agar after 21 days of culture of *S. rebaudiana* on MS medium with 0.25 mg/L BA. Welander et al. (2014) show that Digitalis and Echinacea gained significantly more weight during cultivation in TIS compared to agar medium, while Rubus showed the opposite result. These results might be due to the fact that TIS provides efficient ventilation of the plant tissues due to the intermittent contact with the medium, because most of the time the plant material is not submerged but surrounded with plenty of sterile air. When submerged, there is direct contact between the liquid medium and the leaves; this makes possible nutrient uptake by the leaves, unlike in semisolid media (Jesionek et al., 2017; Vives et al., 2017; Monja-Mio et al, 2021).

SCoT genetic analysis

In total, 15 SCoT primers were used in this study for the initial screening between the field-grown mother plant and in vitro-derived Stevia shoots cultured on MS medium supplemented with different concentrations of BA, in two culture systems, but only ten SCoT primers had amplified clearly and reproducible bands. The number of scorable bands for each SCoT primer varied from 4 (SCoT 9) to 7 (SCoT 18) (Table 2).

Table 2. The level of polymorphism detected with SCoT primers in *Stevia rebaudiana* shoots grown *in vitro* in two culture systems on MS medium supplemented with different concentrations of BA

Primer name	Size of bands (bp)	Number of monomorphyc bands
SCoT 9	300-800	4
SCoT 10	400-1000	6
SCoT 12	400-1800	6
SCoT 15	450-1300	5
SCoT 16	400-1700	6
SCoT 18	400-2000	7
SCoT 19	400-1500	6
SCoT 21	400-800	5
SCoT 22	400-1200	6
SCoT 23	400-3200	5

Each SCoT primer generated amplification products ranging in size from 300 bp (SCoT 9) to 3200 bp (SCoT 23). The results of this study showed that the primers produced amplification products which were monomorphic across all the analysed samples and no polymorphism was detected in in vitro shoots obtained in two culture systems on MS medium supplemented with different concentrations of BA (Figure 5).

Maintaining the clonal fidelity of in vitro-cultured plants is an important aspect of commercial micropropagation (Rai, 2023).

Evaluation of genetic fidelity of in vitro-grown plants is essential before their commercialization, especially in superior genotypes in which the regenerated plants are expected to be true-to-type with their mother plants (Bairu et al. 2011; Krishna et al. 2016; Arora et al. 2022). In the last decade, SCoT markers either alone or in combination with other traditional molecular markers such as RAPD and ISSR have been used to detect somaclonal variation or to assess genetic homogeneity in tissue culture-raised plants of many plant species (Rai, 2023).

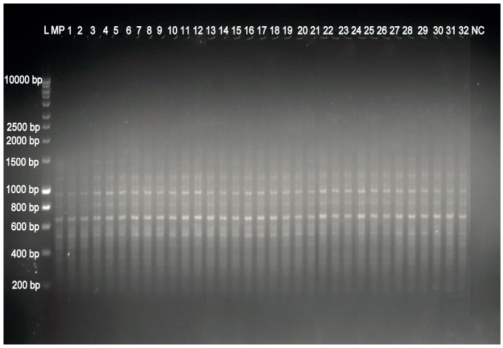


Figure 5. The SCoT profiles of the mother plant and *in vitro* shoots obtained in two culture systems on MS medium supplemented with different concentrations of BA generated by primer SCoT 18. Lanes: MP – SCoT bands from the field-grown mother plant; 1-16 - SCoT bands from shoots grown on solid agar-gellified MS media supplemented with different concentrations of BA (4 shoots x 4 culture media variants); 17-32- SCoT bands from shoots grown on temporary immersion system in liquid MS media supplemented with different concentrations of BA (4 shoots x 4 culture media variants); L - molecular marker (1Kb, Invitrogen, USA); NC - sample controls without DNA

In addition, several DNA-based molecular markers such as RAPD and ISSR markers have been used to assess the genetic fidelity of *S. rebaudiana* of tissue culture-raised plants (Modi et al., 2012; Lata et al., 2013). To our knowledge, the present study is the first report confirming the genetic stability of micropropagated Stevia plants and their mother plant using SCoT markers.

CONCLUSIONS

The experiments presented in this study demonstrate that TIS can be used to increase in vitro biomass production of *S. rebaudiana*. Maximum biomass production (1396.72 ± 54.03 mg/inoculum) was recorded using medium MS with a concentration of 0.2 mg/L BA in TIS. Also, the water content of Stevia biomass tended to decrease in temporary immersion system (TIS) compared to solid agar-gelled MS media (SM).

The genetic uniformity of the *in vitro*-grown shoots with their mother plant was confirmed in both culture systems using SCoT molecular markers, thus revealing the sustainability of the culture systems for *in vitro* biomass production of *S. rebaudiana*.

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RESEARCH ON THE BEHAVIOR OF SOME SWEET POTATO GENOTYPES CULTIVATED ON THE SANDY SOILS OF SOUTHERN ROMANIA

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Abstract

In order to increase the degree of food security in the areas affected by thermohydric stress in Romania, the aim is to identify new species of plants that can optimally exploit the microclimate of the area, through their biological potential. The pedoclimatic conditions recorded in the southern part of Romania offer an optimal microclimate for the growth and development of the sweet potato plant (Ipomoea batatas), which is a thermophilic plant, specific to tropical and subtropical areas. Research carried out between 2020-2022 on the sandy soils of southern Oltenia, showed that the sweet potato genotype s studied behaved differently in terms of tuber quality, accumulating a total amount of dry matter between 27% for the variety JUHWANGMI and 46.98% in the variety HAYANMI, with a variety average of 35.35%. Obtaining competitive productions is influenced by the choice of the most suitable sweet potato genotype for the area of sandy soils in S-W Oltenia. The average production for the three years of the study showed values between 17804 kg/ha for the DCh 19/3 genotype and 53368 kg/ha for the DK 19/1 genotype.

***Key words**: sweet potato, genotypes, sandy soil, production, diseases.*

INTRODUCTION

The sweet potato (*Ipomoea batatas* L.) belongs to the *Convolvulaceae* family, together with flowering plants such as morning glory (*Ipomoea purpurea* and other species of *Ipomoea*) and is related to perennial weeds, such as the swallowtail (*Convolvulus arvensis*), being originally from Central America and northwestern part of South America. The sweet potato combines a number of advantages that make it a plant with an important role in the sustainability of food security, being at the same time a key element for improving nutritional standards and generating sources of income (Ewell P.T., 2002).

The variable nature of the climate in the area of sandy soils, especially the lack of precipitation, as well as the low fertility of sandy soils, determines that the production obtained from some agricultural crops on fairly large areas is greatly reduced. In order to obtain high, safe and stable productions, it is necessary to choose the assortment of plants, the varieties with high adaptability to different climate and soil conditions. In this context, at RDSPCS Dăbuleni, research was initiated regarding the testing of some Korean sweet potato genotypes (*Ipomoea batatas*), using different planting seasons, in order to develop the culture technology and promote this plant in the area. The sweet potato fulfills a number of core roles in the global food system, all of which have fundamental implications for meeting food demands, reducing poverty and increasing food security (El - Sheikha and Ray, 2017). Sweet potato roots have high nutritional value and sensory versatility in terms of taste, texture and flesh color (white, cream, yellow, orange, purple). Varieties high in dry matter (> 25%), white flesh color and firm texture after cooking are preferred by consumers in the tropics. These varieties are known as tropical sweet potatoes. Asian specialty types are purple-fleshed sweet potato varieties with an attractive color and high anthocyanin content. In the United States, the popular type is the orange-fleshed potato with low dry matter content (18-25%), high β-carotene, firm texture and sweet taste. According to HUMAN (1992), sweet potato varieties, depending on the duration of the vegetation period, are divided into: early or precocious (90-120 days vegetation period), intermediate (121-140 days) and late (over 140 days of when planting in the field). Sweet potato (*Ipomoea batatas* [L.] Lam) is a drought-resistant plant with vigorous growth and high productivity, adaptable to sandy soils (Iamandei Maria et al., 2014; Diaconu Aurelia et al., 2016), being the least vulnerable crop to climate change because *Ipomoea batatas* plants grow well at higher temperatures than other crops (Hahn, 1977; Date and Eronico, 1987; Waribo and Ogidi, 2014). The sweet potato is attacked by approximately 300 species of anthropods that can cause severe production losses (Talekar, 1991), but also by more than 30 diseases (Clark & co., 2013; Johnson & Gurr, 2016), and plants infested with *Fusarium oxysporum* has been detected in the culture established at SCDCPN Dăbuleni since 2016 (Boiu-Sicuia et al., 2017). In the conditions of the sandy soils of southern Oltenia, the sweet potato varieties studied behaved differently in terms of tuber quality, depending on the variety and climate conditions. Truong D. et al., 2018, showed that the nutritional composition of sweet potato tubers varies greatly depending on the cultivar, growing conditions, maturity and storage. In general, sweet potato tubers have a high moisture content with an average dry matter content of 25-30%. Tsou and Hong, 1992 and Brabet et al., 1998 reported a wide range of total dry matter content of 13-45% from a collection of sweet potato germplasm and Bradbury and Singh (1986) reported values between 9.5 and 25.0 mg/100 g for ascorbic acid and 7.3-13.6 mg/100 g for dehydroascorbic acid, resulting in a total vitamin C range of 7.3-34.5 mg/100 g for sweet potato roots. There is great variability in sugars between sweet potato genotypes. Truong et al., 1986, found total sugars ranging from 5.6% in a Philippine cultivar to 38% in a Louisiana cultivar by dry weight. Sucrose, glucose, and fructose make up most of the total sugars in sweet potato tubers.

MATERIALS AND METHODS

In order to evaluate the behavior to abiotic factors, research was carried out at RDSPCS Dăbuleni between the years 2020-2022, which aimed at the behavior of 5 sweet potato varieties of Korean origin (KSP 1, KSC 1, YULMI , JUHWANGMI, HAYANMI) and five genotypes, namely DK 19/5, DK 19/4, DK 19/1, DK 19/2, DCh 19/3 in the area of sandy soils in the South of Oltenia. The monofactorial experiment was located on a sandy soil with low fertility, protected with PE mulch, under drip irrigation conditions, according to the randomized block method, with 3 repetitions. The size of the variant was 7.65 m^2 and contained a number of 30 plants, arranged in 3 rows.

Variants studied: A total of 10 cultivars were studied, of which seven semi-late genotypes with white flesh (respectively KSP 1, KSC 1, YULMI, HAYANMI, DK 19/4, DK 19/1 and DK 19/2), two early genotypes with yellow (JUHWANGMI and DK 19/5) and a semi-late genotype with purple flesh, respectively DCh 19/3. The geographic coordinates for the place where the experiences took place are: North Latitude: 43° 48′ 04″, East Longitude: 24° 05′ 31″. The experiment was established by shoots produced in the greenhouse. After March 20, when the soil temperature exceeded 10^0C, the tubers were planted in a double-walled greenhouse, where the temperature and air humidity were controlled, in land treated with the product Basamid G, a soil sterilizer with nematocidal, insecticidal, fungicidal and secondary, herbicidal action. In this sense, the product was applied at the beginning of March when the temperature exceeded 6^0C, in a dose of 5 kg/100 m^2, by spreading on the surface of the wet soil, then being incorporated into the soil with the tiller. To stimulate the herbicidal action and to retain the sterilizing gas as long as possible in the soil, the treated soil surface was covered with PE film until March 21. The tubers were planted in a patterned layer, with double protection under the tunnel. A mixture of black soil + sand + peat was used, in a ratio of 1:1:1. Optimum microclimate conditions were ensured, through repeated watering, protection with transparent PE mulch and ventilation, for the creation of vigorous and good shoots for planting in the field. Planting of the shoots was carried out on May 19, in billoned land, protected with smoky PE foil. The land billoning was carried out with MPB 3

+Steyer, which executes 3 rows of billons in one pass. It was fertilized with N150P80K80, during soil preparation, before plowing, using 250 kg/ha of Ammonium Nitrate and 500 kg/ha NPK 16:16:16. Irrigation was carried out by drip and it was aimed to ensure a minimum humidity ceiling of 75-80% of the range of active humidity, and the harvesting of tubers was carried out after 120 days from planting. Tuber samples were collected 120 days after planting, and the following determinations were made in the laboratory:

1. water and total dry matter (TDM) (%) - gravimetric method;
2. soluble dry matter (SDM) (%) - refractometric method;
3. soluble carbohydrates (%) - Fehling Soxhlet method;
4. vitamin C (mg/100g s,p) - iodometric method;
5. starch (%) - gravimetric method.

RESULTS AND DISCUSSIONS

In the field, the ten genotypes had a shoot growth rate ranging from 1.57 cm/day in the DK 19/5 line to 2.71 cm/day in the KSC 1 variety (Figure 1).

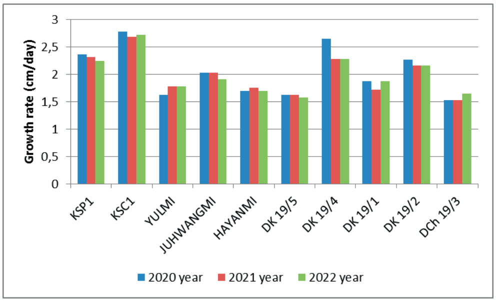

Figure 1. Shoot growth rate in the field (cm/day)

Following the biometric determinations carried out during the experiment 60 days after planting the shoots in the field, the length of the plants had values between 75 cm (at YULMI) and 132.1 cm (at KSC 1), the number of shoots/plant between 4, 6 (at YULMI) and 7.6 at DK 19/5, number of leaves/plant - between 40.65 at YULMI and 66.8 at KSC 1 and petiole length - between 13.3 cm at KSC 1 and 24.7 cm at DK 19/4 (Table 1). Following the harvests to determine the dynamics of tuber weight accumulation, 90 days after planting, the percentage of large tubers had values between 7.40% at KSC 1 and 100% at DCh 19/3.

Table 1. Biometric determinations 60 days after planting in the experimental field

Variant	Plant leght (cm)	Number of shoots	No. Leaves/ plant	Petiole length (cm)	Distance between internodes (cm)
KSP1	115	5.3	56.8	21.3	5.3
KSC 1	132.1	6.3	66.8	13.3	4.6
YULMI	75	4.6	40.65	21.6	4.3
JUHWANGMI	85	5.6	53.65	23.8	7.1
HAYANMI	78	7	48.5	19.6	5.7
DK 19/5	85	7.6	41.3	21.6	4.7
DK 19/4	119	7	46.8	24.7	4.5
DK 19/1	87	7.3	43.5	18.7	4.4
DK 19/2	112	6.3	43.3	16.6	4.2
DCh 19/3	95.1	5.1	43.8	18.7	4.5

The average weight of the tubers high between 0.105 kg/plant at HAYANMI and 0.648 kg/plant at DK 19/1 and plant height was between 0.73 m at DK 19/1 and 2.23 m at KSC 1. At 100 days after planting, the percentage of large tubers of had values between 33.3% at KSC 1 and 70% at DK 19/2, the average weight of large tubers between 0.231 kg/plant at DCh 19/3 and 1.180 kg/plant at DK 19/4 and plant height was between 0, 89 m at DK 19/1 and 2.18 m at KSC1. At 110 days, the percentage of large tubers ranged from 23.07%- at KSP 1 to 100% at DK 19/2, the average weight of large tubers between 0.168 kg/plant at KSP 1 and 1.358 kg/plant at JUHWANGMI and the waist of plants was between 0.93 m at DK 19/5 and 2.51 m at KSP 1. At 120 days, the percentage of large tubers ranged from 40.9 % at YULMI to 91.6 % at DK 19/2, the average weight of large tubers between 0.393 kg/plant at DCh 19/3 and 2.168 kg/plant at DK 19/1 and plant height was between 135 cm at HAYANMI and 271 cm at KSC 1 (Table 2).

Table 2. Gravimetric determinations 120 days after planting

Variant	Large tubers (weight>250 g)				Plant height (cm)
	Percent (%)	Weight (kg)	Diameter (cm)	Length (cm)	
KSP1	43.75	0.680	4.8	19.3	256
KSC 1	52.94	0.990	6.7	31	271
YULMI	40.9	0.930	6	24.3	136
JUHWANGMI	50	0.513	4.6	26.2	176
HAYANMI	46.15	0.575	5.8	24.3	135
DK 19/5	50	0.515	5.4	21.4	152
DK 19/4	81.8	0.596	7.1	21.6	142
DK 19/1	66.6	2.168	7.1	20	139
DK 19/2	91.6	2.075	7.4	15	155
DCh 19/3	44.4	0.393	4.3	18.5	161

The average production estimated after harvest at 90, 100, 110 and 120 days after the final planting of sweet potato shoots in the field, showed a high production potential of the DK 19/1 line compared to the other varieties (Figure 2).

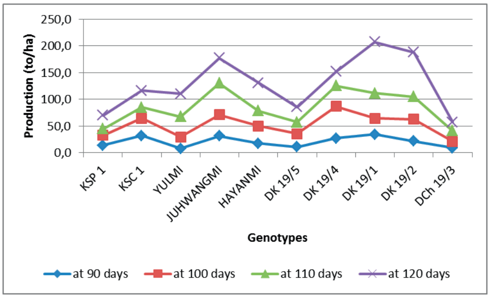

Figure 2. Estimated production at harvests from experience (kg/ha)

The average of the productions estimated after harvesting the experience for each variety separately, at the 3 repetitions, showed values between 15860 kg/ha for the DCh 19/5 genotype and 57640 kg/ha for DK 19/1 (Table 3).

Table 3. Determination of average production between 2020-2022 years

Variant	2020 year (t/ha)	2021 year (t/ha)	2022 year (t/ha)	Average production 2020-2022 (t/ha)
KSP1	17.9	18.2	24.5	20.2
KSC 1	19.2	18.8	16.9	18.3
YULMI	34.7	23.4	32.9	30.3
JUHWANGMI	42.7	63.4	53.9	53.3
HAYANMI	26.5	16.7	21.5	21.6
DK 19/5	23.4	21.8	13.4	19.5
DK 19/4	37.0	62.8	46.5	48.8
DK 19/1	41.1	75.4	43.6	53.4
DK 19/2	27.7	41.2	36.7	35.2
DCh 19/3	16.1	19.6	17.8	17.8

The results obtained by carrying out the statistical calculation on the valorization of experiences placed in randomized blocks, with a single factor, showed a significant difference for JUHWANGMI, DK 19/1 and DK 19/4 compared to the average of the varieties (Table 4).

The high production potential of the JUHWANGMI variety, but also of the DK 19/1 and DK 19/4 genotypes, introduced into culture in 2018, was observed.

The higher the amount of total dry matter (TDM) in the tubers, the lower the amount of water, but sweet potato tubers have a high level of moisture (53.02% in HAYANMI variety and 73% in JUHWANGMI variety). The amount of soluble dry matter (SDM) in sweet potato tubers ranged from 9.6% JUHWANGMI to 12% DCh 19/3, with a cultivar average of 35.35%.

Table 4. The influence of the variety on the production results obtained

Variant	Average of production (to/ha)	Difference		Semnification
		to/ha	%	
Control variant	31.838	Mt	100	
Variant	Average of production (t/ha)	Difference		Semnification
		To/ha	%	
KSP1	20.2	-11.638	63.4	
KSC 1	18.307	-13.531	57.5	
YULMI	30.347	-1.491	95.3	
JUHWANGMI	53.32	21.482	167.5	*
HAYANMI	21.56	-10.278	67.7	
DK 19/5	19.51	-12.328	61.3	
DK 19/4	48.76	16.922	153.2	*
DK 19/1	53.37	21.532	167.6	*
DK 19/2	19.511	-12.327	61.3	
DCh 19/3	17.8	-14.038	55.9	

LSD 5%=15.467 t/ha;
LSD 1%=21.987 t/ha;
LSD 0.1 %=31.836 t/ha.

Varieties with a higher amount of soluble dry matter (SDM) also showed a higher amount of soluble carbohydrates: YULMI (9.46%), DK 19/5 (10.00%) and DCh 19/3 (10.33%) (Table 5). As for starch content, it ranged from 11.10% in the YULMI variety to 14.39% in the DK 19/4 variety, with an average of 12.59%. Sweet potatoes are a source of vitamin C for the human body. Under the climatic conditions of 2020-2022, the amount of vitamin C in the tubers ranged from 8.70 mg in YULMI variety to 11.44 mg in DK 19/4 and DK 19/2 varieties, with an average of 10.11 mg.

Table 5. Biochemical composition of potato tubers according to genotype

Variant	Water (%)	Total dry matter (%)	Soluble dry matter (%)	Soluble carbohydrates (%)	Starch (%)	C vitamin (mg/ 100 g s,p*)
KSP1	62.38	37.62	10.6	9.14	11.55	9.68
KSC 1	60.43	39.57	11.0	9.42	13.21	10.56
YULMI	62.05	37.95	11.0	9.46	11.10	8.70
JUHWANGMI	73.00	27.00	9.6	8.27	13.61	9.68
HAYANMI	53.02	46.98	10.4	8.95	11.72	10.56
DK 19/5	69.27	30.73	11.6	10.00	12.33	10.56
DK 19/4	66.67	33.33	10.8	9.30	14.39	11.44
DK 19/1	70.34	29.66	10.7	8.80	13.25	9.68
DK 19/2	67.38	32.62	10.6	9.10	12.78	11.44
DCh 19/3	61.94	38.06	12.0	10.33	11.92	8.80

Starting with the first decade of July, the field was attacked by pathogens *Alternaria porri*, f. sp. *solani* Neerg. *and Fusarium oxysporum* f.

sp. *sweet potato.* The intensity, frequency and degree of attack (%) with which *Alternaria porri,* f. sp. *solani* Neerg manifested itself in the experimental field between the years 2020-2022 (Table 6) showed the resistance of some genotypes to the disease, respectively DK 19/4 and DCh 19/3.

Table 6. Degree of attack (%) of *Alternaria porri,* f. sp. *solani* Neerg between 2020-2022

Variant	FA (%) for years			Degree of attack (%)		
	2020	2021	2022	2020	2021	2022
KSP1	26.67	23.33	23.33	7.17	8.50	11.6
KSC 1	20.00	13.33	30.00	9.17	7.50	13.1
YULMI	26.67	16.67	23.33	13.33	9.17	12.5
JUHWANGMI	33.33	26.67	33.33	14.50	11.1	15.3
HAYANMI	23.33	13.33	23.33	9.83	5.33	11.1
DK 19/5	33.33	30.00	30.00	15.83	15.0	15.0
DK 19/4	20.00	20.00	30.00	7.70	9.27	8.93
DK 19/1	36.67	36.67	30.00	15.93	14.3	13.3
DK 19/2	26.67	23.33	20.00	11.27	8.27	9.50
DCh 19/3	10.00	13.33	20.00	5.83	6.67	9.50

Frequency and degree of attack (%) with which *Fusarium oxysporum* f. sp. *sweet potato* manifested itself in the experimental field between the years 2020-2022 (Table 7) showed the susceptibility of some genotypes to the disease, respectively DK 19/1 and JUHWANGMI.

Table 7. Degree of attack (%) of *Fusarium* oxysporum f. sp. *sweet potato* beetween 2020-2022

Variant	FA (%) for years			Degree of attack (%)		
	2020	2021	2022	2020	2021	2022
KSP1	23.33	16.67	16.67	8.67	7.83	7.00
KSC 1	13.33	20.00	26.67	6.67	8.33	9.00
YULMI	20.00	16.67	20.00	9.17	8.33	9.17
JUHWANGMI	33.33	26.67	30.00	14.00	10.6	11.5
HAYANMI	20.00	16.67	16.67	7.33	7.00	7.00
DK 19/5	30.00	26.67	26.67	12.83	12.8	10.4
DK 19/4	20.00	23.33	26.67	7.83	9.50	8.50
DK 19/1	33.33	26.67	30.00	14.00	12.5	14.1
DK 19/2	16.67	16.67	16.67	10.00	8.33	7.50
DCh 19/3	13.33	13.33	10.00	5.00	5.83	5.00

CONCLUSIONS

The climatic conditions during the sweet potato vegetation period, recorded during the years 2020-2022 (high temperatures during the period of formation and accumulation of tubers, as well as drought conditions) were beneficial to the accumulation of assimilates in the tubers.
The studied cultivars accumulated an amount of solube dry matter (SDM) between 27% for the JUHWANGMI cultivar and 46.98% for the HAYANMI cultivar, with an average of the cultivars of 35.35%.

The analysis of variance on the yield obtained in the sweet potato genotypes revealed a significant difference for JUHWANGMI, DK 19/1 and DK 19/4 compared to the average yield (31.838 to/ha).
In the field, the ten genotypes had a shoot growth rate ranging from 1.57 cm/day in the DK 19/5 line to 2.71 cm/day in the KSC 1 variety.
Susceptibility to the two diseases in the experimental field between the years 2020-2022, showed the DK 19/5, DK 19/1 and JUHWANGMI genotypes, while the DCh 19/3 genotype showed some resistance.

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DETERMINATION OF THE MINERAL PROFILE OF POTATOES PEEL, BY-PRODUCT FROM POTATO PROCESSING - A PRELIMINARY STUDY

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Abstract

Potatoes play an important role in ensuring global food security. The potato peel, resulting as a by-product from the processing of potatoes, still contains available quantities of nutrient compounds, among which the mineral elements are counted. The purpose of this study is to determine the mineral profile of the potato peel obtained by peeling some local potatoes, in order to use them to enrich the mineral intake of some food products. The total concentrations of Na, K, Ca, Mg, Fe, Mn, Zn and Cu potato peel from potatoes sold in agro-food markets from Timișoara city (Romania) were determined. Preliminary results revealed that 100g of dried potato peel contain appreciable amounts of essential elements: 38.60-82.30 mg/100 g Na, 1856-2531 mg/100 g K, 155-201 mg/100 g Ca, 122-193 mg/100 g Mg, 4.62-10.88 mg/100 g Fe, 0.64-1.22 mg/100 g Mn, 1.61-2.53 mg/100 g Zn, 0.78-1.60 mg/100 g Cu. These values suggest that these by-products, after proper processing, could be introduced into the diet as a source of essential minerals.

***Key words**: potato, potatoes peel, essential minerals.*

INTRODUCTION

Potatoes are one of the main food sources in the world, being a valuable source of starch, proteins, ascorbic acid, carbohydrates, minerals, vitamins and fibers, alkaloids, flavonoids, phenolic compounds and low fat content (Priedniece et al., 2017; Taiyeba et al., 2020). The potato tubers have high concentrations of promoting substances, like ascorbate, carotenoid, and various organic acids and amino acids which enhance the absorption of essential micronutrients. The potato fibers, the potassium, the C and B6 vitamins, together with the lack of cholesterol, all sustain the health of the heart (Nischala, 2019). According to Frida fooddata dk., the proximate composition and mineral content of raw potato (*Solanum tuberosum* L.), shows the following values: 2.00% protein, 17.30% carbohydrate, 1.40% dietary fibre, 0.30% fat, 0.90% ash, 20.50 % dry matter, 7.00 mg/100 g Na, 414 mg/100 g K, 6.76 mg/100 g Ca, 20.40 mg/100 g Mg, 1.03 mg/100 g Fe, 0.05 mg/100 g Cu, 0.30 mg/100 g Zn, 0.23 mg/100 g Mn, 0.60 μg/100 g Cr, 0.27 μg/100 g Se, 55.20 mg/100 g P, 1.20μg/100 g I, 5.75μg/100 g Ni, 0.28μg/100 g Hg, 1.39 μg/100 g As, 2.07 μg/100 g Cd , 0.81 μg/100 g Pb. The proximate and mineral composition depends on potato tissue and cultivar (Vaitkevičienė, 2019). Potato peels, considered waste in many households, as well as in the potato processing industry (Khattak and Rahman, 2017; Nischala, 2019), still contain a series of useful compounds from nutritional and pharmacological point of view, which by different procedures, like extraction or other processes, may be used to produce biofuels, dietary fiber, biofertilizers, biogase and food additives (Verma et al., 2021; Nischala, 2019; Zhivkova, 2021). Potatoes peel contains various polyphenols and phenolic acids (responsible for its antioxidant activities), fatty acids and lipids (showed antibacterial activities); potato peel also contains starch (25%), non-starch polysaccharide (30%), protein (18%), acid soluble and acid insoluble lignin (20%), lipids (1%) and ash (6%) on dry basis (Abebaw, 2020; Eshak and Mousa, 2015). Potato peel waste, processed by various procedures, has numerous practical uses, these being food preservation (as a promising source of natural antioxidant), obtaining antimicrobial ingredients (that could serve as food preservative in food processing industries) and pharmaceutical ingredient, as a source of dietary fiber, ingredient in the baking industry, in the production of a starch hydrolyzing enzyme, thermostable alpha-amylase under controlled solid-state growth conditions (which is widely employed in food industry), is used for the synthesis of biofertilizers, and in animal feed, for production of lactic acid, for biogas productions and as a source of renewable energy (Verma et al., 2021; Abebaw, 2020; Taiyeba, 2020; Thakur et al. 2020; Lau et al., 2021). The data presented in the literature of this field reveal that potato peels contain important quantities of mineral elements, essential for the normal functioning of the human body. The concentrations of some minerals are larger in the skin than in the flesh of the tuber (Verma et al., 2021; Nischala, 2019**)**. Miles et al. (2009) investigated the impact effect of the Zebra Chip disease on the mineral composition of 'Atlantic' potato tubers, for both tuber flesh and peel tissues, from potatoes grown at two separate and geographically distant locations, under controlled cage conditions. The results of this study show that the non-infected potato peels with Zebra Chip disease contain important amounts of mineral elements: 0.11 - 0.14% P, 3.20 - 3.32% K; 0.12 - 0.15% S; 0.38 - 0.93% Ca, 0.22 - 0.23% Mg, 0.03 - 0.07% Na. In a study regarding the variation of the mineral composition within the potato tuber (*Solanum tuberosum* - 'Stirling' variety) planted at Balruddery Farm (Dundee, UK), Subramanian et al. (2011) have observed that the concentrations of most minerals were higher in the skin than in the flesh of tubers; the potato skin contained about 17% of total tuber zinc, 34% of calcium and 55% of iron. The authors of the study show that the mineral concentration in the tuber surface layer shows the following values: 1.90 mg/g Mg, 39.30 mg/g K, 2.20 mg/g Ca, 32.80 mg/g Zn, 17.50 mg/g, 308 mg/g Fe, 10.20 mg/g Cu. Studying the hepato protective effect of potato and apple peels as antioxidant on intoxicated rats with CCl4, Sello (2011) discovered that potato peels contain 18.90% protein, 0.99% fat, 5.30% ash, 4.84% moisture, 69.97% carbohydrate and: 0.83 ppm Cu, 39.20 ppm Ca, 0.52 ppm Zn, 18.6 ppm Fe. Analyzing the feasibility of utilizing peelings from sweet melon, banana and potato in methane production through anaerobic digestion, Jekayinfa (2015) found out that the waste of potato peels used in the production of biogas contain also important quantities of macro and micro essential elements: 24.80 ppm Cu, 22.80 ppm Zn, 178 ppm Fe, 0.05% Mg, 0.04% Na, 0.16% Ca, 3.09% K, 1.80% P (in dry weight). Zoair et al., 2016 have studied the use of orange, banana and potato peels for preparing functional cupcakes and crackers with high fiber content. The authors of this study showed that, in addition to some bioactive components such as dietary fiber and antioxidants, potato peels also contain important amounts of: 59.92 mg/100 g Na, 88.25 mg/100 g K, 161.21 mg/100 g Ca, 9.12 mg/100 g Mg, 1.19 mg/100 g Fe, 0.71 mg/100 g Mn, 1.09 mg/100 g Cu, bioelements with an important role in the functioning of the human body. The peels of vegetables and fruits contain significant amounts of mineral elements demonstrated by, Khattak and Rahman (2017), who in the analysis of potato peels *(Solanum tuberosum)* obtained by peeling potatoes from the local market of Peshawardi (Pakistan), they found the following values: 206±9 mg/100 g Na, 1895±43 mg/100 g K, 73±3 mg/100 g Ca, 142±7 mg/100 g Mg, 11.00±0.00 mg/100 g Fe, 3.00±0.00 mg/100 g Mn, 9.00±0.00 mg/100 g Zn. Various quantities of K, Fe, riboflavin, folic acid and vitamins can be found mainly in the thick periderm of the potato peel. The concentrations of some minerals are larger in the skin than in the flesh of the tuber (Nischala, 2019). Thakur et al. (2020) reported that potato peel contained the highest concentration of K (1823 to 3342 mg/kg DW) followed by Fe (43 to 56 mg/kg DW) and Zn (20 to 29 mg/kg DW). Analyzing the nutritional and mineral composition of wasted potato peels coming from potatoes collected from the local market (Bulgaria), Zhivkova, (2021) has reported that these contained the highest amount of crude protein (2.67%), digestible carbohydrates (9.50%) and water content (84.30%) and important quantities of mineral elements which decreased in the following order: K(59 mg/kg) > S(390 mg/kg) > P(378 mg/kg) > Mg(325 mg/kg) > Ca(130 mg/kg) > Al(62.70 mg/kg)> Fe(41.60 mg/kg) > Na(17.60 mg/kg) > Zn(4.32 mg/kg) >Mn(2.03 mg/kg) > Cu(1.67 mg/kg) > B(1.21 mg/kg). 'Irish' potato peel, a waste by-product of potato processing, is a good source of phytochemicals (flavonoids and terpenes) nutritional components such as starch, dietary fiber, proteins, amino acids, vitamins (B6, B3, C) and mineral elements, particularly K, P, Ca, Na, Mg, Mn, Fe and Zn (Akinsulie et al., 2021). According to Akinsulie et al., (2021) the peels of 'Irish' potato contain: 4.54% crude protein, 1.65% crude fat, 4.81% crude fibre, 3.67% ash, 9.90% moisture, 67.89% dry matter, 0.16% Mg, 0.10% Ca, 0.05% Na, 0.26% K, 0.21% P, 0.87% Cl, 8.50 mg/kg Mn, 12.40 mg/kg Fe. The objective of the present work is the determination of the mineral profile of potato peels resulting as household waste after food preparation and the evaluation of their mineral intake. The total concentrations of Na, K, Ca, Mg, Fe, Mn, Zn and Cu potato peel from potatoes sold in agro-food markets from the city of Timișoara (Romania) were determined.

MATERIALS AND METHODS

To carry out the experiment, three batches of potatoes, *Solanum tuberosum* (approximately 1 kg of potato tubers) were taken from local agrofood markets in Timișoara city (Romania). Five potatoes have been chosen, from each batch, which, after washing thoroughly with a jet of tap water and drying by blotting with filter paper, were peeled using a stainless steel knife. The potato peels obtained were washed again with distilled water, to remove possible impurities, then cut into small pieces and dried in an oven at 60 °C until constant mass. The dried samples were ground in a kitchen grinder and stored in brown jars (at a temperature not exceeding 5°C) until further analysis. The determination of the mineral elements in dried potato peels was carried out by the atomic absorption spectroscopy in the flame method, the calcination variant and the solubilization of the ash in 0.5 N nitric acid (Rada et al., 2018). Practically, 1.0 g dried potato peel was calcined at 550 °C in two rounds of 4 hours each, then the ash obtained was treated with 20 mL of 0.5 N HNO_3 solution, then evaporated until almost dry. This operation was repeated two more times, after which it was added quantitatively with small portions of 0.5 N nitric acid and distilled water to level of 50 mL. Determination of the element concentrations the clear solution brought to the level of 50 mL was carried out using the Varian 280 FS Spectrometer, in the air - acetylene flame. Working parameters of the spectrometer: wavelength, air flow and acetylene, etc. were those recommended by the device supplier. Simultaneously with the measurement of the analyzed samples and under the same working conditions, the solutions used to calibrate the device were also measured. All the mineral composition analyses were performed in triplicate.

RESULTS AND DISCUSSIONS

The results obtained at the determination of the mineral elements for the three batches of investigated potato peels (marked in the text with B1, B2 and B3) are presented in Table 1.

Table 1. Mineral profile of some potato peels (*Solanum tuberosum*)

Specification	Mineral content, mg/100 g on dry weight basis							
	Na	K	Ca	Mg	Fe	Mn	Zn	Cu
Batch 1 (B1)	38.60± 1.07	1856± 13.77	158± 7.79	122± 6.53	4.62± 0.31	0.64± 0.08	1.61± 0.18	1.07 ±0.17
Batch 2 (B2)	82.30± 2.61	2531± 17.56	201± 8.96	193± 7.36	10.88± 0.89	1.22± 0.89	2.53± 0.10	1.60± 0.12
Batch 3 (B3)	44.50± 3.19	2241± 16.32	155± 6.13	148± 7.35	8.22± 0.68	0.71± 0.08	1.88± 0.16	0.78 ±0.06
Mean value	*55.13± 19.36*	*2209± 276*	*181± 28.90*	*154± 29.30*	*7.91± 2.57*	*0.97± 0.26*	*2.01± 0.39*	*1.34± 0.27*

As can be seen from Table 1, the analyzed potato peels, contains important amounts of essential mineral elements unevenly distributed, their concentration being included in wide concentration limits, depending on the origin of the potato peels and also on the nature of the essential element analyzed: 38.60 ÷ 82.30 mg/100 g Na, 1856 ÷ 2531mg/100 g K, 155 ÷ 201 mg/100 g Ca, 122 ÷ 193 mg/100 g Mg, 4.62 ÷ 10.88 mg/100 g Fe, 0.64 ÷ 1.22 mg/100 g Mn, 1.61 ÷ 2.53 mg/100 g Zn and 0.78 ÷ 1.60 mg/100 g Cu (mean values). This is also confirmed by the literature references which show that the chemical composition of potato peels is influenced by a series of factors such as: the variety of potato, pedoclimatic conditions, cultivation techniques, etc. (Vaitkevičienė, 2019). **Potassium** is the main cation in intracellular fluid and functions in the acid-base balance and in the regulation of osmotic pressure (Soetan et al., 2010). This essential microelement is the best represented among all the analyzed elements, its concentration presenting average values between 1856 ÷ 2531 mg/100 g DW. These values are of the same order of magnitude or close to those obtained by Khattak and Rahman, (2017), respectively Thakur et al., (2020) but lower than those reported by Subramanian, et al. (2011) and Jekayinfa (2015). The average concentration of this essential macroelement is 2209±276 mg/kg DW. **Calcium** is an important constituent of bones and teeth, helps regulate nerve and muscle function and is important in blood clotting, blood pressure regulation and immune system health (Soetan et al., 2010). Compared to potassium, calcium was determined in much lower concentrations, the concentration limits of this macroelement having values between 155 - 201 mg/100 g DW. The average concentration of this element in the three batches of potato peels (181±28.90 mg/100 g DW) is close to the average concentration of magnesium and much higher compared to the average concentrations of Na, Fe, Mn, Zn and Cu. Calcium concentrations, determined experimentally, are in agreement with those obtained by Jekayinfa (2015) and Zoair et al. (2016) but higher than those reported by Zhivkova (2021) and Akinsulie et al., 2021. **Magnesium** is an essential macroelement for ATP synthesis, oxidative phosphorylation, protein synthesis, muscular contraction, neuromuscular conduction, bone structure, blood sugar metabolism, membrane stabilization, and DNA transcription (Soetan et al., 2010). The magnesium concentration in the potato peels analyzed shows values between 122 - 193 mg/100 g DW. The average value of the magnesium concentration is lower (154±9.30 mg/100 g DW), but close to the average calcium concentration. Magnesium concentrations in the analyzed potato peels show values comparable to those reported by Subramanian, et al. (2011), Khattak. and Rahman (2017) and Akinsulie et al. (2021), but higher than those reported by Jekayinfa (2015), Zoair et al. (2016) and Zhivkova (2021). **Sodium**, a macroelement that contributes to the regulation of the osmotic balance of all solutions in the body and adjusts the volume of intra/intercellular solutions (Soetan et al., 2010), was determined in much lower concentrations than potassium and lower than calcium and magnesium (38.60 ÷ 82.30 mg/100 g DW). The average value of sodium concentrations in potato peels analyzed are in accordance with those obtained by Miles et al. (2009), Zoair et al. (2016) and Akinsulie et al. (2021), but lower than the value reported by Jekayinfa (2015) and higher than Zhivkova (2021). **Iron** is a component of myoglobin and hemoglobin, functions as a carrier of oxygen in the blood and muscles (Attar, 2020). The concentrations of this essential microelement in the samples of potato peels show average values between 4.62 - 10.88 mg/100g DW. Compared to the other analyzed microelements, iron was determined in the highest concentrations (7.91±2.57 mg/100 g DW). Comparable values of the concentration of some potato peels were also determined by Khattak and Rahman (2017), Thakur et al. (2020) and Zhivkova (2021). Lower values of iron concentration were reported by Zoair et al. (2016), Sello (2011) while Subramanian, et al., 2011 and Jekayinfa (2015) obtained higher values. **Manganese**, an essential microelement that helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones (Attar, 2020). Compared to the rest of the microelements, manganese was determined in the lowest concentrations (0.97±0.26 mg/100 g DW). The Mn concentrations in the potato peels analyzed have values between 0.64 - 1.22 mg/100 g DW. These values are close to those obtained by Zoair et al. (2016) and Akinsulie et al. (2021), but smaller compared to the value reported by

Khattak. and Rahman (2017) and higher than those obtained by Subramanian et al. (2011) and Zhivkova (2021). **Zinc** has a fundamental relevance for many molecular, cellular, metabolic, and immunological processes, including anti-oxidative, anti-inflammatory, and anti-apoptotic responses (Attar, 2020). This essential microelement was identified in smaller amounts compared to iron (1.61 - 2.53 mg/100 g DW), but slightly higher than copper and manganese. The concentrations of zinc in the three batches of potato peels and the calculated average value is included in the range of the values obtained by Jekayinfa (2015) and Thakur et al. (2020). Much lower values, respectively higher values were reported by Subramanian et al. (2011), Sello (2011), Zhivkova (2021), respectively Khattak. and Rahman (2017). **Copper** is one of various fundamental trace metals that are necessary in supporting biological functions for the human organism, forming part of many copper dependent enzymes and proteins (Attar, 2020). This essential micronutrient that is associated in the formation of red blood cells (Attar, 2020) was determined in concentrations between 0.78 – 1.60 mg/100 g DW. The average value of the copper concentration, calculated for the three batches of potato peels (1.34± 0.27 mg/100 g DW) shows that this microelement is present in lower concentrations than Fe and Zn, but slightly higher than Mn. These values are comparable to the value determined by Zoair et al., (2016), but higher than those reported by Subramanian, et al. (2011), Sello (2011) and Zhivkova (2021).

From those presented previously it can be stated that the analyzed potato peels contain important amounts of essential macro and microelements. Taking into account the average concentrations calculated on the basis of the concentrations determined in the three batches of potato peels, it can be observed that the distribution of macroelements in potato peels follows the following decreasing trend: Fe> Zn > Cu≅Mn. The obtained results confirm that the peels have a rich source of minerals and can be used as food or as dietary ingredients after appropriate processing. The average values of the mineral concentration experimentally determined (Table 1) can be used to evaluate the mineral intake of dried potato peels in the recommended daily diet. For the evaluation of the mineral intake, the Recommended Dietary Allowances and Adequate Intakes, Elements Food and Nutrition Board, National Academies - Food and Nutrition Board, Institute of Medicine, for people aged between 31 and 70 years were taken into account (Table 2).

Table 2. Recommended dietary allowance intakes (man and women 31-70 years) and tolerable upper intake levels elements

Specification		Mineral elements, mg/day							
		Na	K	Ca	Mg	Fe	Mn	Zn	Cu
Recommended values	*Man*	1500	4700	1000	420	8	2.3	11	0.9
	Women	1500	4700	1000	320	18	1.8	8	0.9
Tolerable upper levels	*Man and women*	2.300	-	2000	350	45	11	40	10

The obtained results, for the evaluation of the mineral intake, shows that, under the conditions of the present experiment, 100 g of dried potato peel covers different percentages of the required mineral elements recommended (Table 3).

Table 3. Mineral intake in the recommended daily diet, for men and women between 30-70 years corresponding to 100 g of dried potato peels

Specification	Mineral intake, %							
	Na	K	Ca	Mg	Fe	Mn	Zn	Cu
Man	3.68	47.01	18.07	36.75	99.00	63.00	18.24	148.00
Women	3.68	47.01	7.13	48.23	43.93	80.28	25.00	148.00

From those presented in Table 3, it can be seen that a consumption of 100g potatoes peel powder covers 148% of the daily copper requirement - for men and women; 99% and 43.93% of the daily iron requirement - for men and women, respectively; 80.28% and 63.00% of the daily manganese requirement - for woman and men, respectively; 47% of the daily requirement of potassium - for men and women; 48.23% and 36.75% of the daily magnesium requirement - for woman and men, respectively; 25.00% and 18.24% of the daily requirement of zinc, respectively; 18.07% and 7.13% of the daily calcium requirement - for men and women; 3.68% of the daily sodium requirement - for men and women. In addition, we note that the amount of copper contained in 100 g of potatoes peel exceeds the recommended daily copper requirement for men and women between 30-70 years. Therefore, under the conditions of the present experiment, a consumption of 100 g of potato peel, would also bring a surplus of 148% of the Cu requirement. Taking into account the tolerable upper intake levels elements per day, for men and women between 31-70 years (Table 2) it can be said that the increased content of Cu in potato peel does not pose a health risk. The preliminary results obtained in the evaluation of the mineral intake show that the dried potatoes peel obtained under the conditions of the present experiment could be considered as a source of essential mineral elements, especially as a source of Cu, Fe, Mn as well as K and Mg. Potatoes peel powder cannot be considered as a source of Ca and Na. These values suggest that these by-products, after proper processing, could be introduced into the diet as a source of essential minerals (Cu, Fe, Mn as well as K and Mg). In addition, the superior valorisation of this by-product of potatoes can serve as a method of greening of waste potatoes.

CONCLUSIONS

The preliminary results obtained for determination of the mineral profile of the potato peels analysed under the conditions of this experiment show that it contains important amounts of essential macro and microelements. The average concentrations of the analysed macro and microelements show the following decreasing trend: K> Ca> Mg> Na, respectively: Fe> Zn > Cu≅Mn. Potatoes peel powder cannot be taken into account as a source of Ca and Na. Finally, it can be stated that these by-products, after proper processing, could be introduced into the diet as a source of Cu, Fe, Mn as well as K and Mg. In addition, the superior valorization of this by-product of potatoes can serve as a method of greening of waste potatoes.

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A REVIEW OF PLANT-BASED, HIGH PROTEIN SNACK BARS: COMPOSITION, NUTRITIONAL VALUE AND HEALTH BENEFITS

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Abstract

In present days, consumers tend to spend more effort and time in order to find a well-balanced diet for maintaining a healthy life-style despite the daily-life problems that occur because of the current life and work style. Alternative food products such as functional foods or nutraceutical are gaining more sympathy from the general consumers because they can prevent diseases and uphold a healthier life. Food products that are convenient for consumption, storage, and handling are being sought after by consumers. Snack-bars are ready-to-eat nutritious products, that can contain different vitamins, proteins and other components that are of value for the human nutrition, and are also easy to eat. Snack-bars can be used as a meal replacement, as desserts or as salty snacks throughout the day, and their consumption is influenced by different factors such as protein content, sugar content, protein and fibre content or vitamin content. This review aims to highlight the current researches in the development of plant-based, high protein snack bars, and their composition, nutritional value and health benefits.

Key words: *plant-based snack bar, health benefits, functional food, protein content.*

INTRODUCTION

The current work style and the increasing number of families where both partners work have led to a decrease in the time available for cooking and therefore a greater demand for convenience and ready-to-eat food products foods, which should be fresh, natural, minimally processed and without additives (Eldesouky & Mesias, 2014). Snack-type meals, with or without substantial nutritional value, have become an alternative to fast meals, being popular among consumers of all ages (Constantin & Istrati, 2018). Recent changes in the way of shopping preferences and consuming food products have led consumers to give greater importance to aspects such as convenience, sustainability and the health impact of the food products bought. This represents a challenge for the food industry, especially in regard to perishable foods such as fresh fruits and vegetables (Mesías et al., 2021). Therefore, current research directions are directed towards the full use of harvested fruits and vegetables, minimizing the amount of waste resulting from processing them. Food products are recommended to be consumed in their integral form. The more processed a product is, the more it can lose vitamins, minerals or fiber from its composition (Richter et al., 2015). So, achieving a nutritionally balanced protein snack is a scientifically and technologically advanced goal.

The main characteristics of food bars are that they have enough nutritional benefits for the health of the consumer and are also easy to consume. Foods bars are found in the markets in different forms, the main ones being: salty and sweet cereal bars, sweet fruit bars and unsweetened cereal and fruit bars. Because of their main ingredients, they are a good source of fibres (Paiva et al., 2012), energy (Jabeen et al., 2022) and protein.

Although the term functional food is being used for some time in the food industry, there is no official or accepted current definition for it. Functional foods are products that are naturally beneficial for the health of the consumer or have distinct, fortified, and enriched elements that provide health benefits besides those that come from the supply of essential nutrients (Wang & Li, 2015; Sharanya & Penchalaraju, 2016). The main roles of functional foods in promoting human health are presented in Figure 1.

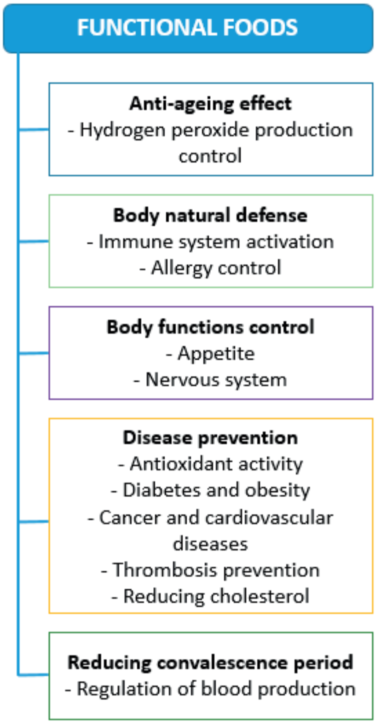

Figure 1. The role of functional foods in regulating processes in the body (source: Constantin & Istrati, 2018)

MATERIALS AND METHODS

This state of the art study is a literature review that investigated reviews and research articles that have been published in the last decade regarding high interest topics such as functional foods products and plant based high protein snack bars.

The content analysed and presented in this review article is focused on highlighting the developments, ingredients, nutritional values and health benefits of ready-to-eat salty snack-bars, which are gaining more and more popularity between food product consumers because of their advantages, such as ease of consumption or high protein values. Several international databases such as, Web of Science, Wiley, Scopus, Elsevier and Springer and Google Academic were electronically scanned for study articles and reviews and the main keywords were: "ready-to-eat snack bars", "high protein content vegetables", "health benefits of vegetables protein".

RESULTS AND DISCUSSIONS

Nowadays, diets that promote the consumption of high amounts of carbohydrates and sugars are linked with higher disease incidence and mortality rates. In opposition, plant based-diets which consist of legumes, vegetables, fruits and whole grains are generally considered to promote human health (Herpich, 2022). Plant-based diets are not only environmentally sustainable, but play a major role in the prevention of several diseases, such as metabolic syndrome (McGrath & Fernandez, 2022) chronic kidney disease (Bernstein, 2007), hypertension, acidosis, diabetes (Betz, 2021), and cardiovascular disease (Herpich, 2022). High-protein bars are popular food products, that contain 20-50% protein and can provide in a quick way the energy that the human body needs at a certain point (Jiang, 2021), thus being consumed all over the world but the manufacturing process can be difficult for the producers because processing issues such as clogging, sticking and cold flow.

An important part of the human diet are proteins, that contribute directly to human health and are usually found in plants (soy, beans, chickpeas, buckwheat) and animals (meat, milk, egg, fish). (Fox et al., 2015; Kumar et al., 2022; Jin et al., 2022; Grasso et al., 2022; Qin et al., 2022). Protein based snack bars usually contain proteins from cereals and dairy, carbohydrates in different quantities, fats and other auxiliary ingredients such as preservatives, flavors and stabilizers (Constantin & Istrati, 2018).

Vitamins, as proteins, serve a critical role in the human nutrition, being needed in order to maintain a healthy life and to guide several metabolic processes (Sá et al., 2022). Vitamins such as B1, B2, B5, E and C can be found in several kinds of snack-bars on the market, the proportions of each vitamin depending on the ingredients of the snack bar (Constantin & Istrati, 2018). Vitamin B1 is usually found in cereals, nuts and whole grains, thus this vitamin is present in higher quantities in oats, peanuts, multigrain snack-bars (Mikkelsen & Apostolopoulos, 2019). Snack-bars rich in oats, soybean, mushrooms and cauliflower are a good source of vitamin B5 (Yadav & Negi, 2021). Vitamin C is found in food products like tomatoes, cabbage, parsley, spinach, red and green pepper, red cabbage, eggplant and broccoli, all of which can be found in snack-bar type of products (Tylewicz et al., 2020). Currently, their industry is developing testing methods, such as wear testing, which will help the problems that occur in the formulation development stage (Sparkman et al., 2019).

Mushrooms

The use of mushrooms as a functional ingredient has increased over the years because of their high protein and fiber content as well as the low fat content. Moreover, they contain valuable constituents such as polyphenols, terpene and terpenoids, mannitol and trehalose which are beneficial for human consumption and health (You et al., 2022). González et al., 2021 studied the possibility of obtaining and characterizing a protein concentrate from a *Pleurotus ostreatus* flour. Nutritional and functional proprieties were evaluated and the parameters for extraction, such as pH and flour-solvent ratio were optimized. The results showed that the protein density increased to 78% and the protein digestibility increased by the decrease of total phenolic compounds in the protein concentrate.

Spim et al., 2021, evaluated the consumer preferences and nutritional value of four different kinds of snack bars, two sweet and two salty one, made from *Lentinula edodes* (shiitake) and other vegetable ingredients. The sensorial tests were performed by hedonic scale analysis of texture, aroma, taste and appearance, and the nutritional values analyzed were moisture content, lipids, fibers, proteins, glucans and phenolic compounds. The results showed that the sweet bars had better sensory acceptance, and as for the nutritional values, the shiitake bars showed high concentrations of calcium, iron, phosphorus, potassium, zinc, manganese, phenolic compounds and glucans.

Peas and beans

Peas and beans are currently used as the main ingredients in vegetables based snacks because of their high protein content. Several studies have been made to study the protein content and the factors affecting this parameter. Walters et al., 2022, studied the changes in the protein content of Field peas (*Pisum sativum*) and Faba beans (*Vicia faba*) cultivated under different ecophysiological factors. Soil composition, meteorological parameters and cultivar conditions were evaluated and the protein content was evaluated. The results showed that the protein content of the analyzed peas and beans was influenced directly by the cultivar and the period of cultivation. In terms of the ecophysiological factors, higher temperatures and low rainfall were associated with a high protein content.

A snack bar from bean and oat flour was developed by Ramírez-Jiménez et al., 2018, and the consumer acceptance and nutritional values (lipids, fibers, carbohydrates, protein, total flavonoids and antioxidant activity) were assessed. In order to carry out the experiments, 27 different samples where the main ingredients varied in percentage were tested and ranked accordingly to the most desired formulations. The results showed that the snack bars made from bean and oat flour had better values in protein content, total flavonoids and antioxidant capacity compared to a control samples that no bean or oat flour added. Also, the carbohydrates and lipid content was lower.

Soy

Soy protein and its by-products are used in the food industry as raw materials in different kinds of salty snacks and food products because of their high-protein content. As soy protein is easily digestible, having a corrected amino acid score of 1.00, almost the same as animal proteins and from a sensory point of view a desirable texture, it can be successfully used as a good source of vegetable proteins by the food industry (Qin et al., 2022).

Lobato et al., 2012, developed a snack bar rich in isoflavones and soy protein as a functional food product in order to help control people with dyslipidaemia. The analysis carries out in the studies focused on the shelf-life of the snack-bars and on the health benefits. The results showed that the water activity and hardness of the developed snack bars increased over the shelf-life analysis. In order to see the health benefits of the snack bars over the high-density lipoprotein cholesterol and triglycerides levels, a clinical trial was carried out by testing lipid profiles of 22 dyslipidaemic subjects for a period of 45 days. An increase in high-density lipoprotein cholesterol (HDL-c) (+8%) and a decrease in the triglycerides levels (-20%) was observed when compared to baselines values.

Other ingredients

da Silva et al., 2014, carried out a study in which marolo (*Annona crassiflora* Mart) flour was used as a functional ingredient in the composition of a snack bar in order to valorise the nutritional proprieties of this fruit. The results showed that the addition of up to 20% of marolo flour in the snack bars increased the dietary fiber, minerals and vitamin C content and also the antioxidant activity. The addition of up to 10% of this flour into the snack bar, improved its organoleptic proprieties such as texture, taste, appearance and overall acceptance.

Rawat & Darappa, 2015, carried out a study in which they created a novel energy bar by replacing brown flour with a fibre rich mixture (banana, coconut and oats flour, psyllium husk) and a protein rich mixture (sesame, chickpea flour, soya and whey protein isolates). The authors analysed the during a 3 months' period the nutritional proprieties (protein and fibre content), rheological and quality characteristics of the energy bars. The results showed that the protein and dietary fibres showed 9 times increase values over the analysis period.

Table 1. Protein bar composition

Protein bar composition	Protein and fibre content (%)	Advantages	References
Dried shiitake, oats, quinoa, rice flakes, flaxseed and sesame seeds, dried tomatoes, peanut butter, parmesan cheese and condiments parsley, garlic, onion, oregano, thyme, bay leaf, pepper and salt	Proteins: 2.27 ± 0.23 Fibres: 9.99 ± 2.00	Health benefits, higher protein and fibre content, low-cost production and food consumer acceptance	Spim et al., 2021
Rice crisps, glucose syrup, honey, vegetable oil, quickcook rolled oats, glycerol, whey protein concentrate, maltodextrin, pectin, sugar, citric acid, glucose syrup, honey, vegetable shortening, glycerol, and apple purée.	Proteins: 2.01 ± 0.07	Health benefits, good source of dietary fibres and polyphenols	Sun-Waterhouse et al., 2010
Water, corn syrup, glycerine, brown sugar, Arabic gum, emulsifier, puffed wheat, wheat germ, commercial coatings, shortening, soy nuggets, soy protein, soy fibre, vanilla extract	Proteins: 3.00 % Fibres: 13 %	High protein and fibre content and B-complex vitamins	Aramouni & Abu-Ghoush, 2011
Sugar, glucose syrup, palm oil, glycerol, pastry flour, oats, golden raisins, apricot, apple, pear	For pear enriched samples: Proteins: 5.07 ± 0.03 / Fibres: 8.06 ± 0.50 For apple enriched samples: Proteins: 5.19 ± 0.09 / Fibres: 7.59 ± 0.55	High fibre content, good sensory proprieties	Bchir et al., 2018
Cooked bean flour, oat flour, water and soy lecithin as emulsifier in different proportions	Proteins: 12.46 ± 0.05 Dietary fibres: 20.84 ± 0.08	Improved the nutritional and bioactive profile; Protein, dietary fibre and antioxidant capacity increased values	Ramírez-Jiménez et al., 2018
Dates, cheddar cheese, whey protein isolate), roasted chickpea flour, and rice flour in different proportions	Proteins: 22.3% to 23.6% Crude fiber: 5.81 to 7.16	Improved the total protein content and diversity of proteins	Jabeen et al., 2022

A high protein content snack bar made from modified sweet potato flour, mung beans flour and whey protein concentrate was developed by Sunyoto et al., 2019. The scope of the study was to developed a snack bar with a protein content of at least 10-15% in order to meet emergency food requirements. The best formulation was 55:45 ratios of sweet potato flour:mung beans flour, thus obtaining a product with a protein content of 13.96% and raw fiber content of 3.45%. Also the sensory analysis showed that the consumer acceptance was good, the color, flavor and aroma being accepted.

Several research articles studied the potential use of different cereals and vegetables in obtaining high nutritional value snack bars, such as: corn bran (de Sousa et al., 2019), jerivá flour (Silva et al., 2016), date (Ibrahim et al., 2021), sorghum flakes (Ribanar et al., 2015), banana flour (Umme et al., 2021), jackfruit seed flour (Meethal et al., 2017)

CONCLUSIONS

In recent years, the food industry is slowly leaning towards developing food products that are rich in nutrients, easy-to-eat and possess health promoting ingredients. One category of foods that cover all these aspects and they also have a long shelf-life are snack-bars, which can be found on the market in different forms: sweet and salty snack-bars. Usually, snack-bars are consumed throughout the day and because of their nutritional benefits (high content of proteins, fibers, minerals or vitamins) they can be considered good meal replacements. There has been a lot of research studies that showed that snack bars can be classified as functional foods with good promoting health proprieties, as functional ingredients such as bean flour, dried shiitake mushroom and quinoa flour or fibre rich mixtures (banana, coconut and oats flour, psyllium husk), protein rich mixture (sesame, chickpea flour, soya and whey protein isolates), are incorporated in the recipe.

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PRELIMINARY DATA REGARDING THE APPLICATION OF *BACILLUS* SPP. IN THE CULTURE TECHNOLOGY OF PROTECTED TOMATOES IN ORDER TO INCREASE PRODUCTIVITY IN THE BUCHAREST AREA

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Abstract

Now the current trend in agriculture around the world deals with promoting growth and controlling crop diseases and pests organically by reducing or even eliminating the application of chemicals. In the present study, soil and foliar biostimulating products containing Bacillus spp. (Bactilis, Leaf Power, NitroStim and Rizobac) were tested in the greenhouse on three tomato hybrids in order to increase production through a more environmentally friendly technology. The following parameters were monitored: stem diameter in the lower part, the plants height, the sequence of the inflorescences, the number of flowers and fruits on the plant and on the inflorescences, the percentage of fruits on the plant and in inflorescences, the production of fruits on the plant, fruits firmness, total dry matter, total soluble solids, titratable acidity.The results showed that, the application of Rizobac on Kingset and Bucanero hybrids, led to an increase of the number of inflorescences and fruits.. The size of the fruit was most positively influenced by the application of Bactilis fertilizers, the increase being between 6-15% depending on the hybrid. The hybrid-fertilizer combination is important and influences the production obtained.

Key words: *biological, fertilizers, microorganisms, production, PGPR, vegetables.*

INTRODUCTION

Tomatoes (Lycopersicon esculentum Mill) have their origins in South America and, compared to other vegetable species, have the largest cultivation area in the world, being very appreciated by consumers.

The fruits are rich in vitamins, minerals, amino acids and pigments (Dinu, 2017; Soare, 2015) and poor in calories.

Fruits are characterized by high lycopene content, carotene, calcium, magnesium, phosphorus and other elements (Filgueira, 2013), antioxidants (Kalogeropoulos et al., 2012), contributing to prevention of cardiovascular diseases and cancer (Gong et al., 2006) being considered very healthy for the human body.

It is consumed in a wide variety and has a beneficial impact on human health mainly due to the high content of lycopene, folic acid, ascorbic acid, flavonoids, α-tocopherol, potassium and phenolic compounds (Erba et al., 2013).

Tomatoes react very well to various technological interventions, from physical solutions such as more stems to increase yield or the application of biofertilizers, arginine and cysteine (Hoza et al., 2012, 2013, 2018, 2019 and 2022; Apahidean, 2021; Becherescu, 2019).

In recent years, farmers are increasingly interested in good agricultural practices, switching to organic farming and using water and nutrients efficiently. In order to achieve higher quality production there are formulations with bacteria that act as biopesticides, biostimulants or biofertilizers, to increase plant productivity but also to reduce plant residues, thus contributing to consumer protection (Basu, 2021; Chojnacka, 2015), bacteria it is also known for their ability to produce lytic enzyme involved in plant pathogenic inhibition and plant growth. Bacillus spp. was noted both for its positive effects on existing microorganisms in the soil but also for a better assimilation of nutrients, which led to increased productivity by facilitating the absorption of mineral nutrients (Kalam, 2020; Sicuia, 2015).

Bacillus spp., promote plant growth through a better uptake of nutrients such as nitrogen and phosphate, and/or by the production of phytohormones such as auxins, enzymes such as ACC deaminase or volatile organic compounds (VOC) such as 2.3-butanediol and acetoin.

(Asari, 2016; 2017; Borriss, 2016; Fazle Rabbee & Baek, 2020).

Bacillus spp. also improves the main nutrient transport functions from the soil to the plant by solubilizing insoluble zinc compounds and increasing the bioavailability of zinc in the soil (Mumtaz et al., 2017).

Balderas-Ruíz et al., 2021, in theirs study observed that application of *Bacillus* spp. in high concentration influenced tomatoes marketable quantity. The results showed the potential of Bacillus spp. to boost tomato production within the expected range for greenhouse cultivation. Due to the yields and the quality of the fruits obtained, was estimated that the profitability of the treatment was 2.5 times higher than the non-fertilized one.

The application of treatments with *Bacillus* spp. has been shown to be useful during the post-harvest storage period, the application of treatments with Bacillus spp. has been found to delay senescence in cherry tomatoes (Zhu et al., 2021).

In conclusion, *Bacillus* spp. stood out both for its positive effects on existing microorganisms in the soil and for a better assimilation of nutrients, which led to increased productivity and their use can optimize the use of synthetic fertilizers in agriculture (Dragomir & Hoza, 2022).

MATERIALS AND METHODS

The experiment took place in the Research Greenhouse of University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV), during 2022. The experiment was organized in randomized blocks, with three replicates on each experimental variants and six plants/repetition, on an area of 200 m^2, with two variable factors, one factor was, the tomato hybrids and the other factor was growth biostimulators.

The main purpose of the experiment was to observe and monitor the main quantitative and qualitative characteristics of hybrids grown in the presence of biostimulating products: Bactilis 5 L/ha, Leaf Power 5 L/ha, NitroStim 5 L/ha and Rizobac 10 L/ha, the control variant was without biostimulats application.

It was used two plant growth stimulating products applied to the soil, respectively Rizobac and Bactilis and foliar, respectively NitroStim and Leaf Power. Two foliar fertilizations were carried out with NitroStim, the first application was made before the appearance of the first inflorescence, and the second one 14 days after it. Fertilization with Leaf Power, Rizobac and Bactillis a wasere carried out eight times during the vegetation period.

Bactilis is a microbial inoculant containing beneficial bacteria in the form of endospores. As soon as Bactilis is applied in the soil, spores quickly germinate and the bacteria that occur start proliferating and colonizing the plant root. In parallel, bacteria start producing substances-metabolites which improve rooting, plant growth and vigour and also improve the resistance of the root system in stressful conditions caused by various biotic and environmental factors (Retrieved from https://www.humofert.gr/en/product/2015-05-29-12-29-20/bactilis-detail.html).

Leaf Power is a composite product that combines the properties of a growth stimulant, an organic foliar fertilizer and a microbial inoculant. Leaf Power contains beneficial microorganisms in the endospore form which exist naturally on the leaves, shoots and the rhizosphere of most plant species (Retrieved from https://www.humofert.gr/en/product/2015-05-29-12-29-20/leaf-power-1-detail.html).

NitroStim is a microbial solution which stimulates plants' growth thanks to the activity of specific beneficial nitrogen fixing bacteria, which are capable of penetrating into the above-ground plants parts (phyllosphere) and of becoming endofytes. Nitrogen fixing phyllosphere endofytes fix atmospheric nitrogen and convert it into a readily assimilable by plants form, ensuring a fast, vivid and balanced growth (Retrieved from https://www.humofert.gr/en/product/2015-05-29-12-29-20/nitrostim-detail.html).

Rizobac is a microbial inoculant, rich in nutrients and beneficial microorganisms which enhances rooting, achieves a rapid establishment of the transplanting crops in the soil and increases the penetration and expansion of the roots for all crops. Rizobac contains beneficial soil bacteria in a total population of 1x1011 cfu (colony forming units) per liter, which create a healthy soil environment, promote the root system growth, contribute to the better plant nutrition and fortify plant resistance against various biotic and abiotic factors. Furthermore, the rich in nutrients substrate of Rizobac, stimulates the microbial activity and contributes to the creation of a strong and voluminous root which supplies and supports the vigorous growth of the plant. Rizobac is suitable for crops cultivated according to the standards of organic, integrated and conventional farming (Retrieved from https://www.humofert.gr/en/product/2015-05-29-12-29-20/biostimulants-1/rooting/rizobac-1-detail.html).

The biological material used was represented by three F1 hybrids, Bucanero, Buffalosun and Kingset.

By combining the 2 factors, 15 variants resulted, each variant having 3 repetitions and 6 plants per repetition, as follows: V1 - Bactilis + Bucanero F1, V2 - Bactilis + Buffalosun F1 , V3 - Bactilis + Kingset F1, V4 - Leaf Power + Bucanero F1, V5 - Leaf Power + Buffalosun F1, V6 - Leaf Power + Kingset F1, V7 - NitroStim + Bucanero F1, V8 - NitroStim + Buffalosun F1, V9 - NitroStim + Kingset F1, V10 - Rizobac + Bucanero F1, V11 - Rizobac + Buffalosun F1, V12 - Rizobac + Kingset F1, V13 - Unfertilized + Bucanero F1, V14 - Unfertilized + Buffalosun F1, V15 - Unfertilized + Kingset F1.

Sowing in order to obtain seedlings was carried out on February 15, their replanting after 14 days from germination, and planting in the greenhouse on April 12, at distances of 0.8 m/0.4 m, resulting in 3.2 pl/m2 and 32,000 pl/ha, the age of the seedlings being 56 days.

Flowering in the first inflorescence started about 21-22 days after planting, and the first fruits appeared after 26-27 days. In the following inflorescences, flowering took place at 36-37 days in the second inflorescence, 46-47 days in the third inflorescence and after 55-56 days in the fourth inflorescence. Fruit set was after 41-42 days in the second inflorescence, 51-52 days in the third inflorescence and after 60-61 days in the fourth inflorescence. Fruit harvesting started on July 22.

During the vegetation period, the specific care works to this crop were applied.

When the plants started to grow, measurements were made on stem diameter in the lower part, the height of the plants until the cutting of the growth tip, the sequence of the inflorescences, the number of flowers and fruits on the plant and separately on the inflorescences, the percentage of fruits set on the plant and in inflorescences, the production of fruits on the plant. The tomato plants were led with four inflorescences. At the end of the culture, measurements were made on the length of the stem, the weight by weighing and the volume of the root system of the plants, using a 1 L Class A graduated cylinder, in all experimental variants. Also, determinations were made on the tomato fruits firmness with an electronic penetrometer TR was used with a penetration of Ø 8 mm piston, the average weight by weighing the fruits of each variety and dividing by total number of fruits, four times during harvest, production of tomatoes per hectare, total dry matter by gravimetric method, total soluble solids (% Brix) were determined from tomato juice with refractive device Kruss DR301-95 (% Brix). The titratable acidity (TA) acidity was determined by titration with 0.1N NaOH to pH 8.1. The results been calculated using the following formula and expressed as percentages of citric acid content (Saad et al., 2014):

Percent of titratable acidity = (V x N x 100 x 0.0064)/m, where N is the normality of NaOH, 0.0064 is the conversion factor for citric acid, V is the volume of NaOH used (mL) and m is the mass of tomato sample used (g).
The determinations were made in the Research Center for Studies of Food Quality and Agricultural Products - University of Agronomic Sciences and Veterinary Medicine of Bucharest, laboratories.
Results were interpreted using F-test ($p \leq 0.05$) and T-test ($p \leq 0.05$).
Data values were measured from six replicates and analysed by an F test to establish equal ($P > 0.05$) or unequal ($p < 0.05$) variance and to establish differences of statistical significance the T test $p > 0,05$ not significant, $p < 0.05$ weakly significant, $p < 0.01$ moderately significant, $p < 0.005$ highly significant, $p < 0.001$ very strongly significant $p < 0.0001$ not significant.

RESULTS AND DISCUSSIONS

The application of biostimulating products on the three analyzed hybrids had beneficial effects on the growth and fruiting processes of tomatoes.
Thus, on stem diameter in the lower part varied between 14.28 mm and 10.95 mm. Fertilization caused better growth in all hybrids, the differences in diameter being due to fertilization and less to the hybrid used (Figure 1).

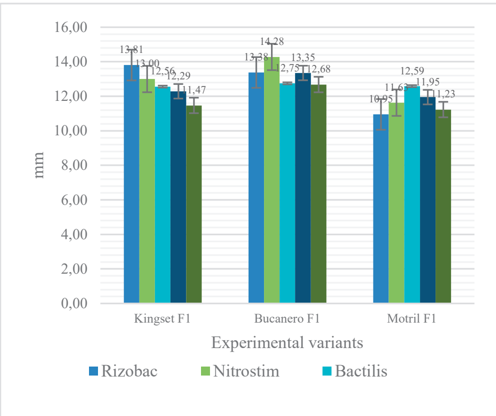


Figure 1. Influence of fertilization on tomato hybrids on the stem diameter in the lower part of the plants, mm

The significantly positively influenced hybrid ($P=0.017$) was Kingset when Rizobac fertilizer was applied, with an average plant diameter of 13.81 mm (Figure 1).
The height of all hybrids was not significantly influenced by fertilizer application. The highest plant growth in height was in the Kingset hybrid, reaching 145.83 cm when applying Leaf Power, while by applying Bactilis products, NitroStims and Rizobac the plant growth had fairly close values (Figure 2).

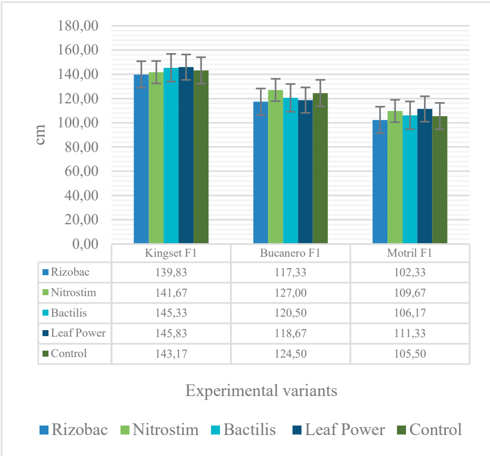


Figure 2. Influence of fertilization on tomato hybrids on height of plants, cm

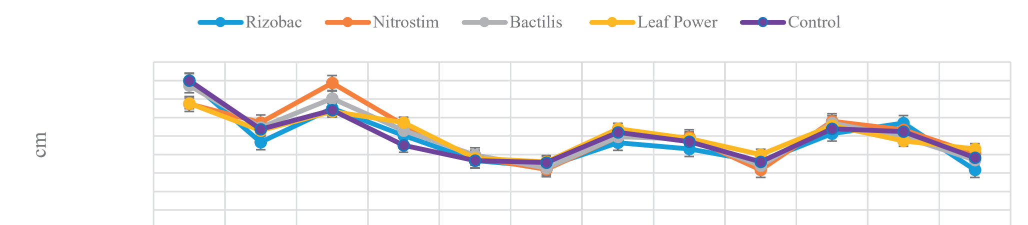
In the Figure 3, can been observe the height of the plants when the third inflorescence appears.

Figure 3. Tomates culture in the greenhouse (USAMV)

Regarding the insertion of the inflorescences on the stem, the application of biofertilizer NitroStim ($P = 0.009$) and Leaf Power ($P = 0.017$) on the hybrid Kingset, led to the insertion at 33.67 cm from the base, respecttively 33.83 cm compared to the unfertilized variant with 40 cm from the base (Figure 4).

On insertion of the first inflorescence, the only negative value, statistically ensured was when NitroStim was applied to the Motril hybrid, which recorded 39.33 cm compared to the control with 32 cm. The insertion of the second inflorescence was significantly better when NitroStim was applied (p = 0.026) for the Motril F1 hybrid with a value of 16 cm compared to the unfertilized version of 17.83 cm.

In the case of the insertion of the third and fourth inflorescences, the differences were not as great as in the previously analyzed parameters, the ferlilizants had a rather small influence, not statistically ensured (Figure 4).



	Kingset F1	Bucanero F1	Motril F1	Kingset F1	Bucanero F1	Motril F1	Kingset F1	Bucanero F1	Motril F1	Kingset F1	Bucanero F1	Motril F1
Rizobac	40,00	23,33	32,50	25,17	18,33	16,67	23,17	21,50	18,00	25,67	28,50	15,83
Nitrostim	33,67	28,67	39,33	28,00	19,17	16,00	24,83	24,67	15,83	29,00	26,67	20,17
Bactilis	38,67	27,33	35,17	26,50	19,83	16,33	24,83	24,17	17,00	28,50	24,83	18,50
Leaf Power	33,83	26,50	31,67	28,67	19,00	18,00	27,00	24,33	20,00	27,83	23,67	21,50
Control	40,00	26,83	32,00	22,50	18,33	17,83	26,00	23,50	18,00	27,00	26,17	19,17

Figure 4. Influence of fertilization on tomato hybrids distances between

The productive capacity of the tomato plants was influenced by the hybrid, but also by the application of biofertilizers, which led to a visible improvement in the number of flowers, the number of fruits and the fruit set percentage. In the plants belonging to the Kingset F1, when applying Rizobac, the average number of flowers in inflorescence I increased from 5.8 flowers in the control, to 9.4 flowers and the fruit set percentage was very significantly higher (P = 0.008). The number of flowers in inflorescence IV increased from 7.6 flowers to 10.8 flowers/inflorescence (P = 0.002) (Table 1). The influence of Rizobac can also be seen in the total number of flowers/plant of 34.2 and the number of fruits/plant of 18 compared to control, one of 22.6 flowers and 12.8 fruits respectively. The differences being strongly significant P = 0.003 and very strongly significant P = 0.001. Under the conditions of this experiment, the highest production of flowers/plant was recorded when NitroStim fertilizer was applied, 34.8 and the number of fruits/plant was 16.2 (Table 1).

Table 1. The number of flowers, fruits and the fruit set percentage in the Kingset hybrid, on fertilization variants

Fertilizers	Kingset F1										
	Inflorescence I		Inflorescence II		Inflorescence III		Inflorescence IV		Total		
	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Total fruit	% set fruit
Rizobac	9.4	3.4	7.4	4.4	6.6	4.8	10.8	5.4	34.2	18	53.32 %
T test	0.034	0.008	0.359	0.524	0.106	0.053	0.002	0.191	0.003	0.001	0.765
NistroStim	6.6	2.2	9	4.2	9.6	4	9.6	5.8	34.8	16.2	47.58 %
T test	0.517	0.251	0.079	0.792	0.063	0.201	0.095	0.243	0.0047	0.011	0.317
Bactilis	6.2	2.8	4.8	3.8	5.6	3	6.4	3.8	23	13.4	60.50 %
T test	0.778	0.453	0.656	0.856	0.369	0.809	0.246	0.849	0.907	0.812	0.742
Leaf Power	5	3	4.4	2.6	3.6	1.2	5	2.6	18	9.4	53.32 %
T test	0.475	0.056	0.522	0.025	1.000	0.032	0.023	0.230	0.059	0.047	0.637
Control	5.8	2	5.6	4	3.6	2.8	7.6	4	22.6	12.8	57.07 %

The application of Leaf Power led to a weaker fruit set in inflorescence II (P = 0.025) and Inflorescence III (P = 0.032) and in inflorescence IV the number of flowers was lower than in the control, of 5 flowers respectively 7.6 flowers. The total number of fruits per plant was also influenced by 9.4 fruits/plant compared to 12.8 fruits/plant for control.

In the case of the fruit set percentage, the differences were not so great, the influence being rather small, not statistically ensured (Table 1). Regarding the Bucanero F1, the number of flowers in inflorescence IV was positively influenced by the application of Rizobac fertilizer, as well as fruit set. In inflorescence II, a better influence of Bactilis fertilizer was recorded, with 6.6 flowers and 5 tied fruits, instead in inflorescence IV, a slight decrease (P = 0.048) in the number of flowers can be observed compared to control (Table 2).

The application of NitroStim (P = 0.034) and Leaf Power (P = 0.008) led to a weaker fruit set in inflorescence III, 2.2 and 1.6 respectively comparison with control 3.6 fruits.

The total number of flowers, fruits and the percentage of binding on the plant did not register considerable differences, the influence being quite small. not statistically ensured (Table 2).

Table 2. The number of flowers, fruits and the fruit set percentage in the Bucanero hybrid, on fertilization variants

Bucanero F1											
Fertilizers	Inflorescence I		Inflorescence II		Inflorescence III		Inflorescence IV		Total		
	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Total fruit	% set fruit
Rizobac	5.6	4.8	4.6	3.6	4.8	3.8	5.6	3.2	20.6	15.4	76.21 %
T test	1.000	0.535	0.587	0.667	0.846	0.784	0.046	0.029	0.340	0.213	0.787
NistroStim	6.2	5.4	4.4	3.6	3.8	2.2	3	1.8	17.4	13	76.74 %
T test	0.305	0.724	0.521	0.545	0.467	0.034	0.195	1.000	0.794	0.493	0.903
Bactilis	6.4	4.4	6.6	5	5.8	3.6	2.6	1.6	21.4	14.6	68.67 %
T test	0.252	0.242	0.014	0.040	0.442	1.000	0.048	0.667	0.205	0.656	0.211
Leaf Power	4.4	4	3.4	3	2.6	1.6	3.2	2	13.6	10.6	73.76 %
T test	0.343	0.362	0.760	0.842	0.108	0.008	0.308	0.694	0.151	0.202	0.714
Control	5.6	5.2	3.8	3.2	4.6	3.6	4	1.8	18	13.8	77.71 %

The application of the products on the Motril hybrid, recorded the lowest values in the experiment regarding the number of flowers in inflorescence I and II, but also the total number of flowers on the plant when applying Bactilis and Leaf Power (Table 3).

Table 3. The number of flowers, fruits and the fruit set percentage in the Motril hybrid, on fertilization variant

Motril F1											
Fertilizers	Inflorescence I		Inflorescence II		Inflorescence III		Inflorescence IV		Total		
	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Fruit Set	Total flowers	Total fruit	% set fruit
Rizobac	6.2	2.6	8	4.2	7	4	6.8	4	28	14.8	55.27 %
T test	0.010	1.000	0.620	0.557	0.796	0.764	0.070	0.147	0.515	0.326	0.050
NistroStim	7.4	1.2	4.8	3.6	4.4	1.8	6.8	3.8	23.4	10.4	44.49 %
T test	0.071	0.156	0.027	0.861	0.175	0.205	0.339	0.340	0.080	0.472	0.217
Bactilis	6.2	3.2	3.2	2.6	3.4	2.2	5	2.8	17.8	10.8	62.47 %
T test	0.003	0.494	0.008	0.506	0.078	0.301	1	0.829	0.012	0.570	0.017
Leaf Power	6.4	2.4	5.2	3.2	5.6	3.8	4.8	2.8	22	12.2	55.73 %
T test	0.041	0.766	0.044	0.870	0.359	0.885	0.792	0.803	0.0497	1.000	0.0004
Control	9.4	2.6	9.4	3.4	7.6	3.6	5	2.6	31.4	12.2	38.09 %

The percentage of fruit set was higher when the product was applied and for two of them it was significantly higher Bactilis and Leaf Power of 62.47% and 55.73% in comparison with control of 38.09% (Table 3).

The firmness was not influenced by the application of the products, the recorded values being similar and insignificant from a statistical point of view. The highest value was for the Motril F1, reaching 12.05 N when applying NitroStim and the lowest value was for the Bucanero F1 reaching 4.47 N when applying Leaf Power (Figure 5).

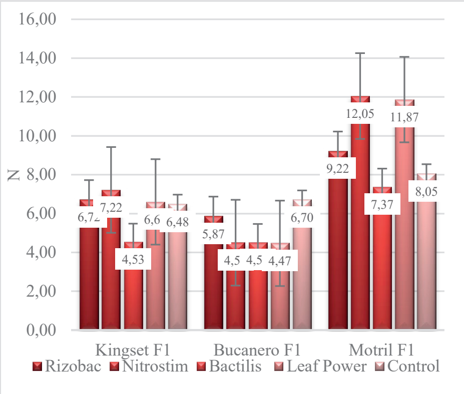
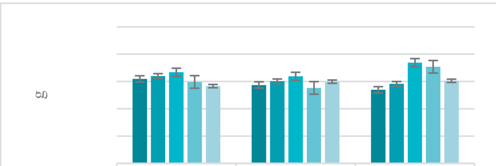


Figure 5. Effect of fertilization on the firmness (N) of tomatoes

Regarding the average weight of the fruits, the differences were not as great as in the previously analyzed parameters.



	Kingset F1	Bucanero F1	Motril F1
Rizobac	154,70	143,65	134,74
Nitrostim	160,11	150,31	145,64
Bactilis	166,85	159,40	184,47
Leaf Power	149,18	138,22	176,93
Control	141,57	149,65	151,23

Rizobac Nitrostim Bactilis Leaf Power Control

Figure 6. Average weight of tomatoes varieties (g)

The fertilisants had a rather small influence, not statistically ensured. The average fruit weight was between 141.57 g and 166.85 g for the Kingset F1, between 138.22 g and 159.40 g for the Bucanero F1 and between 134.74 g and 184 g for the Motril F1 (Figure 6).

Maximum fruit production was achieved when tomato plants were fertilized with Rizobac and increased by 27% for Kingset F1 compared to the unfertilized control version. Bucanero F1 response better to Bactilis with 11% more that control and hybrid Motril to Leaf Power with an increased by 15% more that control.

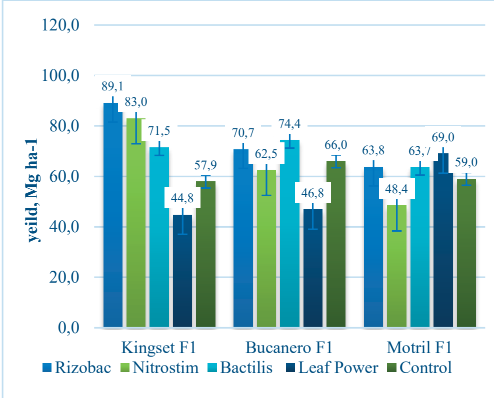


Figure 7. Influence of fertilization on tomato average production, (Mg ha^{-1})

The total dry matter content, regarding the Kingset hybrid, was influenced by the application of the following products: NitroStim recorded a value of 4.94%, Bactilis 5.23% and Leaf Power recorded the value of 5.03%, all values being significantly higher than the unfertilized value of 4.62% (Table 4).

Table 4. Analysis total dry matter content in three tomato hybrids (DM%)

	Total dry matter (DM %)		
	Kingset F1	Bucanero F1	Motril F1
Rizobac	5.05	5.30	4.40
T test	0.107	0.661	0.0005
NistroStim	4.94	5.00	4.83
T test	0.0444	0.1155	0.7570
Bactilis	5.23	4.91	4.69
T test	0.001	0.135	0.116
Leaf Power	5.03	5.78	4.81
T test	0.025	0.082	0.883
Control	4.62	5.39	4.82

Regarding the Bucanero F1 and Motril F1 the values obtained after applying the products were similar and insignificant from a statistical point of view (Table 4).

Sugar content was not influenced by the application of the fertil, the recorded values being similar and insignificant from a statistical point of view (Figure 8). The values obtained was between 4.80% and 3.90% being in accordance with those obtained by (Dobrin et al., 2019).

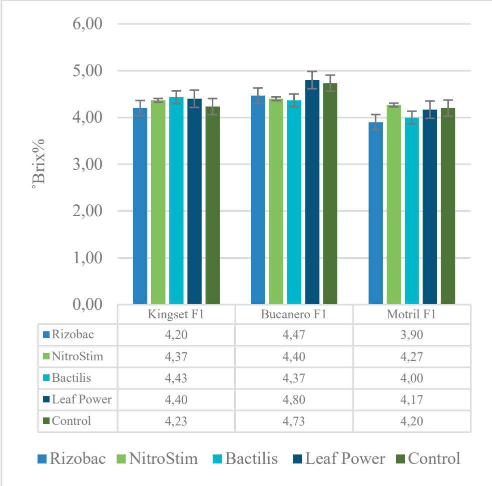


Figure 8. Effect of fertilization on sugar content (°Brix%)

The application of the Rizobac fertilizer led to considerable changes in terms of total titratable acidity for all the hybrids used, ensured from a statistical point of view, thus the Kingset F1 had a higher acidity than its unfertilized version and the Bucanero F1 and Motril F1 had a lower acidity than their unfertilized variants (Table 5).
The NitroStim fertilizer had for Bucanero F1 total titratable acidity = 0.30% lower than the variant unfertilized with 0.36 % (Table 5).
Bactilis, had better results regarding the Kingset F1, compared to the unfertilized version.

Table 5. The effect of applying biofertilizers on the total titratable acidity (% citric acid)

Total titratable acidity (% citric acid FW)			
	Kingset F1	Bucanero F1	Motril F1
Rizobac	0.298	0.296	0.303
T test	0.001	0.002	0.004
NistroStim	0.318	0.291	0.245
T test	0.000	0.001	0.373
Bactilis	0.324	0.350	0.308
T test	0.001	0.261	0.000
Leaf Power	0.361	0.329	0.370
T test	0.000	0.106	0.058
Control	0.276	0.343	0.351

Regarding the root growth parameters the Bactilis fertilizer stands out positively and statistically significantly in the case of all analyzed parameters root length, root weight and root volume. The root length being greater for the Kinset F by 12.1 cm compared to the non-fertilized version. the root weight was greater in Kingset F1 and Bucanero F1 by 17.75 g. respectively 5 g compared to the non-fertilized version and the volume of the roots was more high when applying the fertilizer compared to the non-fertilized version. thus Kingset F1 with 15.83 cm^3. Bucanero F1 with 3 cm^3 and Motril F1 with 4.83 cm^3.

Table 6. Length, weight and root volume

	Root length (cm)			Root weight (g)			Root volume (cm^3)		
	Kingset F1	Bucanero F1	Motril F1	Kingset F1	Bucanero F1	Motril F1	Kingset F1	Bucanero F1	Motril F1
Rizobac	42.03	43.72	37.42	19.51	25.30	20.59	24.67	29.67	25.83
T test	0.007	0.723	0.102	0.175	0.789	0.316	0.477	0.505	0.001
NistroStim	31.28	46.42	25.75	14.97	32.33	18.31	19.17	32.33	16.67
T test	0.204	0.306	0.179	0.044	0.265	0.816	0.552	0.194	0.167
Bactilis	38.43	41.42	35.33	40.82	31.39	18.35	38.00	29.33	17.50
T test	0.008	0.448	0.343	0.003	0.001	0.726	0.003	0.009	0.010
Leaf Power	34.00	37.42	37.48	21.35	22.93	19.70	23.33	22.83	19.17
T test	0.100	0.466	0.272	0.630	0.453	0.422	0.795	0.538	0.0496
Control	26.33	41.42	31.50	23.07	26.39	17.66	22.17	26.33	12.67

CONCLUSIONS

The present research showed that genotype has a large influence and is manifested specifically for all traits related to productivity. Regarding on stem diameter in the lower part, fertilization with Rizobac gave the best results for Kingset hybrid compared to the control. About the insertion of the inflorescences on the stem, the application of biofertilizer NitroStim and Leaf Power on the hybrid Kingset, led to a better insertion, compared to the unfertilized variant.

The application of Rizobac fertilizer to the Kingest hybrid give rise to an increased number of flowers from 5.8 in the control, to 9.4 flowers and the fruit in the first inflorescence also set percentage was very significantly higher which led to an increased productions by 27% more than control variant.
The total dry matter content, regarding the Kingset hybrid, was influenced by the application of NitroStim which recorded a value of 4.94% and Leaf Power recorded the value of 5.03%, all values being significantly

higher than the unfertilized value of 4.62%.The NitroStim fertilizer had for Bucanero F1 total titratable acidity = 0.30% lower than the variant unfertilized with 0.36%.

Regarding the root growth parameters the Bactilis fertilizer stands out positively and statistically significantly in the case of all analyzed parameters root length, root weight and root volume.

The results obtained demonstrated that biofertilizers application on tomato crops in controlled environment, can be a sustainable and organic technology in reducing or even eliminating the application of conventional chemicals.

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OPTIMICATION OF SCHEMES FOR REPRODUCTION OF STEVIA VARIETY 'STELA'

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Abstract

In Agricultural Institute - Shumen it has been carried out maintenance and multiplication of the first Bulgarian stevia variety 'Stela', certified in 2017. In vitro technics for maintenance and micropropagation are used, as well as in vivo development of seedlings from cuttings by preservation and reproduction of rhizomes. During the last years are often the conditions of warm and continuous autumn with a regime of the short day from the end of September, which favors the reproductive development of seeds. The results for the measured indices of height, number of stems, fresh and dry mass and the calculated randeman and dry leaves yield of seed off-springs are indicative for the stabilization of comparatively high levels of productivity of elite clones of the variety 'Stela'. The assessment shows that the most effective scheme for practical realization of seedlings is by sowing seeds in controlled conditions in January-February, diving off the germinated and stabilized plants in April-May, transplanting in June-July and receipt of formed rhizomes in September-October, which, after preservation to be realized during the next period.

Key words*: stevia, variety maintenance, reproduction.*

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial, cross-pollinating plant from the *Asteraceae* family. It is native to the highlands of Paraguay and Brazil (Lewis, 1992). In recent years, there has been an increased interest in its cultivation as a source of natural, non-caloric sweeteners. They belong to the family of diterpene glucosides such as rebaudiside A, B, C, E and stevioside and are up to 300 times sweeter than sucrose (Geuns, 2004).

Due to its sensitivity to low temperatures in countries with a temperate climate, it is grown as an annual and can be maintained by storing the rhizomes (Lankes & Pude, 2008). Propagation by rooting cuttings from rhizomes stored in the winter is widely used. An alternative method is propagation and maintenance by in vitro methods, in which the original genotype is also preserved (Krumov et al., 1984).

In natural conditions, it is propagated by seeds. Ste via is a short-day plant and fertilization requires a certain light and temperature regime (Zaidan et al., 1980). As a cross-pollinated crop, self-incompatibility mechanisms prevent self-pollination and the production of homozygous offspring (Nakamura et al., 1985). Flowering is non-simultaneous and long-lasting. Already in the 1980s at the Sugar Beet Institute - Shumen, research was conducted on the introduction and cultivation of stevia, and the optimal technological conditions were established and methods for in vitro propagation were developed (Varbanov et al., 1996).

With the renewal of interest, the Shumen Agricultural Institute is working on the selection of new forms of stevia. In 2017, the first Bulgarian variety was recognized, with high economic qualities and adapted to the conditions of Bulgaria (Uchkunov, 2006).

Some aspects of obtaining and germinating seeds in vitro and under external conditions have been studied (Bozhimirov & Slavova, 2011; Kikindonov and Enchev, 2012). Under our conditions from propagated in vitro and after prolonged adaptation in spring in greenhouse conditions, individual plants begin to flower at the end of July. The mass flowering is from September until the harvesting of the rhizomes before the formation of autumn frosts (Bozhimirov & Slavova, 2011). The great genetic diversity of the seed progeny is the basis for the enrichment of the gene pool for the selection of forms with high productivity and stevioside content (Caneiro et al., 1997). The research presents the results of attempts to optimize the schemes for the reproduction of seedlings of the stevia variety Stella in the conditions of Bulgaria.

MATERIALS AND METHODS

The data for the survey are from researches in 2017-2021 at the Agricultural Institute - Shumen. Field trials of seedlings from elite branches were conducted on carbonate chernozem, with 50 cm inter-row spacing and 20 cm within-row spacing. Optimum moisture storage is provided with drip irrigation. Pesticide treatment and mineral fertilization are not applied.

Seedlings from rhizomes, cuttings and seeds are planted after 25.04 to avoid frosts. Plants for economic evaluation are measured for height and number of main stems and individually mowed at the appearance of flower buds, before flowering. After separating the leaves from the stems, their mass was measured in the fresh state and after drying. Thus, the weight of the fresh and dry mass, the dry content and the yield as a ratio of the dry leaves to the total fresh mass are determined.

Selected elite plants are left to flower and produce seeds. Individual plants begin to bloom at the end of July. Mass flowering is from September until the harvesting of the rhizomes before the formation of autumn frosts. In our research, two ways of obtaining seeds are used. In the first, individual ripe seeds are harvested from plants in the field. In the second case, whole plants are harvested at the end of the growing season and dried whole. Later, the seeds are threshed without separating the empty and unripe ones.

After taking into account the diseased and dead plants, the rhizomes are harvested at the end of the growing season - usually the beginning of November and stored in a dark room at 0-4°C. In the spring before planting, the surviving rhizomes with the presence of viable stem primordia are counted.

Elite rhizomes are placed under optimal conditions of lighting, humidification and temperature of 25°C for the growth of stems. Cuttings are cut from them into 2-4 times and planted in a soil mixture under the same conditions for rooting.

To determine the laboratory germination, the seeds were planted in harmonica filter paper, in four replicates of 20 seeds, for each origin. They are counted on the seventh day. The mass of 1000 seeds was determined. In a thermostatic room at 20-25°C and lighting for 18 hours in pots with a soil mixture 4/1/1 of peat, perlite and sand, 50 mg of seeds are sown in 4 repetitions for each origin. The seeds are distributed evenly on the surface of the pre-moistened mixture and pressed tightly against it. Optimum humidification is maintained with a polyethylene chamber during the first days, avoiding over moistening.

Rooted plants from seeds and cuttings, usually 30 days after planting and sowing, are plucked - replanted 1 in a pot. Usually, it takes up to 45 days to get a seedling suitable for planting in outdoor conditions.

Statistical data processing included analysis of variance for mean, coefficient of variation, mean error, and trial accuracy.

RESULTS AND DISCUSSIONS

The main method of reproducing stevia in the conditions of Bulgaria with a moderate climate and alternation of seasons is to harvest the developed rhizomes in the fall, store them in the winter and plant them in the spring of the fall year. To maintain a base population of the Stella variety, up to 500 rhizomes are planted annually. During the period 2017-2019, the number of healthy rhizomes that survived from the stored rhizomes in the spring, planted in the field, harvested and removed in the autumn was recorded consecutively. In this way, the proportion of survival during storage and cultivation of perennial rhizomes of elite branches of the variety was calculated (Table 1).

On average over the three years, 15% of the planted rhizomes fall off during the growing season and are not harvested for foliage and storage. During winter storage and after removal and spring harvesting due to decay and lack of viable stems, an average of 26% is lost.

Propagation by rhizomes leads to an accumulation of soil pathogens over the years and increases the incidence of foliar diseases. This necessitates a mandatory cyclical alternation of propagation by rhizomes with *in vitro* and *in vivo* propagation for the production of elite seedlings.

Table 1. Results of assessment for survival during storage and vegetation of rhizomes from elite branches of stevia variety Stela, 2017-2019

Variants	Stored rhizomes		Planted rhizomes	
	Number	Survived, %	Number	Harvested, %
203/2	150	56.2	78	95.8
A 8	148	75.0	108	89.3
9 E	149	50.0	69	97.4
97 ШЗ	240	50.0	126	100.0
10 ШЗ	273	68.0	95	91.4
7 E	156	76.0	111	93.9
№ 3	150	74.5	101	72.7
№ 5	150	61.8	50	66.0
75 ШЗ	145	83.3	99	87.2
214/2	140	65.0	120	80.0
A 9	239	79.5	125	92.0
A 10	151	80.0	121	86.7
Total	4045		2688	
Mean		73.8		85.8

In 2020, 12 selected rhizomes were placed in sand and under optimal conditions for development with 25°C and regular humidification. After the development of shaped stems of 10-15 cm, the upper parts with up to 4-6 leaves are cut off and planted 8-12 in pots with soil mixture for rooting. Within a month, the branches from the cut rhizome stems form new stems suitable for cuttings. On average, 96 cuttings with 26, 45 and 26 pieces from each cutting were obtained from a rhizome with three cuttings. After 30 days, on average, 85.8% of the cuttings are rooted and plucked - 1 plant is replanted in a pot and after 30-45 days of adaptation to external conditions, seedlings. Suitable for planting under field conditions are obtained.

The effect of the method of propagation by cuttings can be greatly increased by cutting off the tops of the rooted plants to obtain additional cuttings, which, however, is associated with an extension of the period for the production of seedlings.

Table 2. Results of the assessment for reproductive potential of cuttings from stored rhizomes from elite branches of stevia variety Stela, 2021

Variants	Planted cuttings:			Rooted cuttings %
	First dare	Second date	Third date	
9 E	30	45	20	93.8
75 ШЗ	28	46	25	73.5
№ 5	18	42	15	85.0
10 ШЗ	25	35	15	84.1
A 8	31	47	24	88.5
28 CP	20	24	17	91.1
97 ШЗ	15	25	15	86.7
62 ШЗ	36	45	23	90.5
10 ШЗ	33	26	14	94.4
№ 3	1K2	24	12	86.3
№ 4	17	25	15	85.1
A 10	40	43	24	75.7
X	26.7	44.6	26.2	84.9
VC	22.1	18.9	25.3	8.67
P%	4.22	3.11	5.73	2.71

The trend for a longer and warmer autumn in our area in recent years is an opportunity to cover the conditions of the stevia for the accumulated temperature sum and the short day at the end of the growing season. This is a condition for obtaining a larger number of viable seeds. In our research in 2020, two ways of obtaining seeds are used. In the first, individual ripe seeds are harvested from plants in the field. In the second case, whole plants are harvested at the end of the growing season and dried whole. Later, the seeds are threshed without separating the empty and unripe ones.

The seeds obtained in both ways were evaluated for germination under laboratory conditions (Table 3). The difference in germination is significant in favor of the selected single seeds during the growing season, which have an average of 55.7% germination. The postharvest chaff of whole plants at the end of the growing season was 21.8% on average, and with a lower degree of variation VC - 22.6%. The advantage of the second method is obtaining more seeds with significantly fewer resources. This allows more mass use of seeds for reproduction in practice. For this reason, after determining the mass of the seeds, they are sown in a soil mixture of 50 mg per repetition, taking into account the germinated sprouts. With an average mass of 400 mg per 1000 seeds and relatively little variation, an average of 125 seeds per set replicate is obtained and the exact germination rate is easily calculated - an average of 36%.

The results presented are positive for the use of seeds to increase the potential for reproduction in practice. But a strict selection of the elite plants for seed reproduction must be observed to preserve the genotype of the base population of the variety.

Table 3. Results of assessment for qualities of seeds from elite plants of stevia variety Stela, 2021

Variants	Laboratory germination of selected seeds, %	Laboratory germination of seeds after threshing, %	Mass of 1000 seeds, mg	Germination in soil substrate, number of seedlings/50 mg seeds
1. 20-20	81.3	6.70	350	54.3
2. 20 -9	66.7	26.7	354	47.5
3. 20 -6	53.3	33.3	320	51.2
4. 20 - 11	66.7	26.3	350	57.0
5. 20 - 14	40.0	13.7	360	38.0
6. 20 - 15	46.7	13.3	450	39.2
7. 20 - 10	19.8	10.0	430	17.0
8. 20 - 18	73.3	33.3	490	50.0
9. 20- 16	46.3	20.0	400	42.1
10.20 - 19	80.0	33.3	510	66.0
11.20 - 12	53.3	26.7	390	47.3
12.20-5	40.0	20.0	350	31.0
Mean	55.7	21.9	403,6	45.01
SE+/-	12.6	14.8	78.9	12.7
VC%	29.3	22.6	8.73	27.8

In 2020, rhizome seedlings, cuttings and seeds were planted under field conditions. Table 4 shows the results of the analysis of individual assessment by morphological and economic indicators. The rhizomes show the strongest development of the vegetative mass, reflected in taller and more branched stems and a greater accumulation of fresh and dry biomass. Seedlings from cuttings are more vigorous in development than seed progeny, which is due to the slower initial development from germination to obtaining the adapted seedling. According to economic indicators such as dry content and yield, the differences are insignificant. The identical and relatively low levels of variation are also impressive. This is an indicator that the implementation of breeding schemes does not affect the stability of the phenotype of the variety.

Table 4. Results of variation analysis of morphological and productive parameters of harvested plants of elite branches from rhizomes and cuttings, and seeds progenies of Stela variety, 2020

Variants	Height	Stems	Fresh mass from		Dry mass from		Dry matter, total %	Rande man,
			Leaves	Stems	Leaves	Stems		%
	cm	number	g	g	g	g	%	%
640 rhizomes								
Mean	78,2	9,55	274,5	201,8	76,8	56,3	28,1	16,3
max	95,0	19,00	360,0	280,0	105,0	85,0	32,6	21,7
min	65,0	3,00	180,0	120,0	55,0	35,0	20,9	11,9
vc	11,8	29,4	18,8	25,3	20,6	19,9	11,7	17,5
p%	3,56	8,89	5,69	7,63	6,22	9,04	3,55	5,29
515 pcs seedlings from cuttings								
Mean	72,3	5,27	255,9	160,4	70,0	46,8	27,6	16,8
max	85,0	12,0	380,0	260,0	115,0	70,0	31,6	21,4
min	65,00	5,00	150,0	85,00	35,0	25,0	23,9	14,0
vc	9,00	35,0	20,5	22,4	24,4	24,8	9,7	14,7
p%	2,71	10,5	9,21	9,78	10,3	10,5	2,9	4,44
550 pcs of seedlings from seeds								
Mean	71,9	5,91	182,2	126,3	54,0	36,8	30,0	17,8
max	94,0	9,00	210,0	155,0	65,0	50,0	46,8	25,5
min	60,0	3,00	125,0	100,0	45,0	25,0	22,7	13,6
vc	13,0	26,7	14,6	17,2	19,5	22,1	10,9	19,3
p%	3,94	8,05	4,43	5,20	3,49	6,67	6,32	5,82

The possibility of obtaining developed seedlings from seed to compensate for slower initial development has been investigated. For this purpose, non-pricked seedlings from seeds were planted later on 15. 06. densely at an average of 50 plants per square meter. It was harvested and the leaf mass yield was calculated. At the end of the growing season, 1,800 rhizomes with a weight of 10-15 g were obtained from 35 square meters, suitable after storage for planting the following year. The harvested dry leaf mass from this area is 9.17 kg. which equates to 2700 kg per hectare and significant additional income.

Table 5. Results of the evaluation of yielding annual rhizomes from seed for seedlings and the additional production in dense cropping

Variants	Area	Number of plants	Economic Indices	
			Dry leaves Total	Dry leaves Yield
	Sq.m	number	g	g/m2
1. 20-20	3,90	174	1395	356
2. 20 -9	1,75	97	510	292
3. 20 -6	2,75	126	1490	542
4. 20 - 11	3,25	154	675	208
5. 20 - 14	5,05	277	500	100
6. 20 - 15	3,50	185	615	175
7. 20 - 10	3,15	161	760	241
8. 20 - 18	6,30	315	1245	198
9. 20- 16	2,00	119	620	310
10.20 - 19	2,65	115	490	185
11.20 - 12	2,60	78	685	263
12.20-5	0,75	15	185	247
Total	34.95	1816	9170	-
Mean of m^2	1.00	52	-	269.2

CONCLUSIONS

Propagation by rhizomes leads to an accumulation of soil pathogens over the years and increases the incidence of foliar diseases. This necessitates a mandatory cyclical alternation of propagation by rhizomes with *in vitro* and *in vivo* propagation for the production of elite seedlings.

Propagation by cuttings is an effective method of mass production of seedlings, but it is associated with necessary resources for implementation.

The evaluation shows that the most effective scheme for the practical implementation of seedlings is by sowing seeds under controlled conditions in January-February, growing the sprouted and stabilized plants in April-May, transplanting in June-July and obtaining formed rhizomes in October, which after storage to be realized in the next period.

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STUDYING THE INFLUENCE OF BIOLOGICAL FERTILIZATION ON RADISHES IN UNHEATED POLYETHYLENE GREENHOUSES

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Abstract

In an experiment with greenhouse radishes grown according to biological production technology during the period 2018-2020, the influence of the variety, the cultivation scheme, the method of sowing and the applied biological fertilizers on the morphological characteristics and productivity of the crop was evaluated.In scattered sowing, the mass of the whole plant, the mass and the diameter of the root crop were positively affected by the applied biological fertilizers. The yield of ties per unit area has increased to a greater extent in the Edri red variety compared to the Saxa variety. On the other hand, the Large Reds variety has from 12.9% to 23.7% higher average yield in all fertilizing options compared to the control, and in the Saxa variety from 7.3% to 13.2%. In scattered sowing, the highest yield was reported when fertilizing with Italpolina of 8718 connections/dec in the Large Reds variety and 6918 connections/dec in the Saxa variety. The influence of biological fertilization in row sowing is more pronounced and has been proven statistically in both varieties and in all fertilization options. Row sowing gave a higher yield with 11.3% in both varieties. As with scattered sowing, the Edri red variety has a 15.9% to 24.9% higher yield with all fertilization options. The highest productivity was obtained for variant fertilizing with Italpolina in the Large Reds variety 9950 bunch/ha-1 and in the Saxa variety 8906 bunch/ha-1.Organic fertilizers did not lead to the accumulation of excessive amounts of nitrates in both varieties, but varietal differentiation was confirmed. In scattered sowing, they were from 1118.4 mg/kg to 1407.3 mg/kg in the Saxa variety and in Large Reds from 1023.7 mg/kg to 1131.4 mg/kg. Nitrates in row sowing are lower and vary from 969.7 mg/kg to 1051.8 mg/kg for Large Reds variety and for Saxa from 1118.4 mg/kg to 1407.3 mg/kg

Key words*: radishes, greenhouses, organic fertilization, nitrate.*

INTRODUCTION

The concern for the protection of human health is directly related to the production of clean and healthy food. Vegetables occupy an important place in a healthy diet. They are especially valuable during the winter season, when some are grown in greenhouses. In addition to the main vegetables, some cold-resistant crops such as radishes are grown in the autumn-winter and winter-spring period. The product part of them develops in the soil and is strongly influenced by mineral nutrition.

There are a number of studies on the influence of mineral fertilization on the growth, yield and quality of this crop. Under greenhouse conditions, the influence of different forms of nitrogen NH4-N and NO3-N on some parameters of radishes is determined. At the beginning of the growing season, the yield increases with increasing NO3-N in the soil. As NH4-N increases, the yield decreases. Nitrates are the highest when fertilized with NO3-N (Kováčik, P., at all 2004). In similar studies, the influence of nitrogen fertilization was monitored at three doses of 0; 15 and 30 kg N ha^{-1}. The best values for the monitored biological and morphological indicators were found at 15 kg N ha^{-1} (Pedó T. et al., 2014) When vermicompost was used as a source of N, the radish yield was more strongly affected by NO3-N compared to NH4-N. At the higher doses, with an increase in nitrate nitrogen, the content of free nitrates in root crops increases and vitamin C decreases (Kováčik P. et al., 2021). Studies have also been conducted to establish the influence of different forms of K on the quality of root crops. The influence of different sources of K on the quality of root crops when fertilizing with potassium chloride, potassium nitrate and potassium phosphate is evaluated. They established a good response of radishes to K regardless of the source of fertilization. (Maia P. de M. E. et al., 2011) Similar studies with three doses of nitrogen 0.3, 0.6 and 0.9 g.dm^{-3} and two types of potassium fertilizer KCL and K_2SO_4 were carried out in two varieties of radish Carmen and Sinieźka. A differentiated reaction of the varieties was established. Weight and yield in Carmen decreased with increasing nitrogen doses, while in Sinieźka it increased with the higher dose. The form of potassium does not affect the indicators, but when fertilizing with K_2SO_4 there are lower nitrates in the root crops (Nurzyńska-Wierdak, R. et al., 2013). The reaction of greenhouse radishes to the shortage of N.P.K and S was also investigated. The strongest adverse effect is the deficiency of N. (Kováčik, P. and Jančovič, J., 2001).

The subject of research is organic and organo-mineral fertilization. The impact of different types of manure alone or in combination on growth, yield and quality was investigated. (Kowalski, A., Kaniszewski, S 2017); (,Singh, V.,at all 2016); (Bodkhe, V. A., at all 2010); (Mahorkar, V. K., at all 2007); and (Costa, C. C., at all 2006) . Very good results were obtained after fertilization FYM50% +PM50%. High yield was obtained after application of vermicompost. A strong stimulating effect on vegetative growth was found in the combined use of vermicompost in combination with California worm extract.(Araújo, R. at all 2020) The influence of cow manure 20,30, 40 t/ha and poultry manure 10,15,20 t/ha and qualitative and quantitative indicators were tracked. The effect of various sources of organic matter (cattle manure, pig manure, aviary bed) on lettuce cultivation was monitored under greenhouse conditions. No knock-on effect has been found in growing radishes.(Bonela, G. D., at all 2017) When using vermicompost alone, the best results were obtained at a concentration of 40% and 60%. Similar results were obtained by other authors with a combination of peat with vermicompost. (Alsiņa, I.,at all 2013) Comparing mineral and organic fertilization in growing radishes is also the subject of research. The highest values for growth and yield were obtained after fertilization with NPK followed by the organic fertilizers PM, GM, SS, FYM (Mehwish Kiran et al., 2016) In an experiment with three varieties of radish and five types of fertilizers, the best results were obtained with PM and with a combination of PM + NPK. There are similar results with FYM (Subedi S., 2018) Integrated fertilization with organic and mineral fertilizers has shown the best results on growth and yield with increasing levels of fertilization (Verma U. K., et al., 2017) Studies have been conducted with some microbial fertilizers involving *Penicillium* sp. and *Aspergillus* sp. related to phosphorus nutrition. It was found that there were no major differences in phosphorus uptake between treated and untreated plants (Narloch C., 2002) A comparison was made between microbial and organic fertilizers: FYM, phosphobacter Azospirillium, vermicompost, humus acid and NPK. The best root crops in terms of size and weight were recorded when fertilizing with vermicompost (Vijayakumari B. et al., 2012)

Special attention in research is also devoted to the question of the accumulation of nitrates in the root crops of radishes. In an experiment with 10 varieties grown outdoors and in a greenhouse, the tendency to accumulate nitrates was evaluated. The Cortund variety shows the lowest accumulation of nitrates 1158.7 ppm and the highest in Helios 1876.7 ppm. In polyethylene greenhouses, the nitrate content is higher than outdoors by 20-30% (30). In another study, 5 varieties are studied. It is indicated that nitrates are below the MPC of 600 ppm in the Rudolf variety - 293 ppm, Soro - 326 ppm, Hellex - 407 ppm. Above the permissible norms are Altex - 614 ppm, Mirabeu - 689 ppm(Soare R. et al., 2010).

Dates, schemes and methods of sowing are also the subject of research (Bleyaert P., 1990); (Kobryń J., 1987). 3 sowing dates 1, 15 and 30 October and 3 distances 5 cm, 7.5 cm and 10 cm /3 cm ensuring a density of 666 were studied; 444 and 333 ras./m2. The best results were obtained with sowing 1.10 and scheme 10 cm /3 cm. 3 types of seed drills were tested in glasshouse and PE greenhouse conditions: mechanical single-row: 5-row with perforated discs and 15-row pneumatic. The experiment was carried out with two varieties in glasshouse and one variety in PE. It has been established that the pneumatic seeder gives 2 times more yield than the others.

From the literature review, it is clear that different types of fertilizer have different effects on the quality and yield of radishes. The variety has a decisive influence, also the type of fertilizer and the rate of fertilization. The sowing scheme and the density have an influence. The results obtained, even with similar fertilization, vary and are strongly influenced by the variety and growing conditions. This motivated us to experiment with PO with local and adapted radish varieties. In this regard, we set ourselves the goal of exploring different organic fertilizers and seeding schemes suitable for organic production.

MATERIALS AND METHODS

The experiment was conducted in the Educational-experimental field of the Department of Horticulture at the Agrarian University - Plovdiv during the period 2018/2020. A field experiment was conducted with radish culture in an unheated polyethylene greenhouse on the block method in four repetitions with a reporting plot size of 1.4 m^2. Two varieties of radishes Saxa and Large Reds were used. Sowing was done at the beginning of November at a seeding rate of 1.5 g/m^2. The preparation of the soil was carried out in the autumn, and the entire quantity of the tested biological fertilizers was brought in with the pre-sowing preparation of the soil. The following biological fertilizers were tested: Biosol, Italpolina, Vita organic and were applied in the recommended rates. Two methods of sowing were studied: manual - scattered and row sowing with a single-row seeder. The following options were chosen: 1- control (unfertilized); 2 - Biosol 100 kg/ha^{-1}; 3 - Italpolina 25 kg/ha^{-1}; 4 - Vita organic 100 kg/ha^{-1}. Harvesting began when about 10% of the plants formed standard root crops. The harvests were initially carried out once a week and after the second week twice a week. Biometry was done three times to determine the morphological indicators of the plants during the harvesting period. The following indicators were determined: mass of the whole plant, average mass of the root crop, number of leaves per plant, diameter of the root crop. Only the standard yield from plants with standard roots that were healthy and not cracked was counted. The yield was determined as the sum from all bunches during the harvest period. Nitrates in the root crops were counted once, and one bunch was taken for each variant of the four replicates. Standard cultivation technology was applied, but without the use of chemicals. Watering is done by rain.

The obtained results were subjected to statistical processing using the ANOVA statistical software product. Nitrates were determined in the certified chemical laboratory of AU - Plovdiv.

RESULTS AND DISCUSSIONS

One of the tasks we set ourselves was to follow the influence of the studied factors (biofertilizers and sowing method) on basic morphological indicators in relation to productivity and quality. We tested the reaction of two local radish varieties and the possibility of growing them as organic production.In the experiment with applied row sowing with a seeder, very good values were obtained for all biometric indicators. For the Saxa variety, the plants fertilized with Italpolina have the greatest mass, both in the first and in the second year and on average for the study with statistical evidence of differences in GD 5%. (Table 1). The applied fertilization did not have a significant impact on the number of leaves. In the fertilized variants, the plants formed between 10.04 and 10.42 leaves against 10.05 for the control and the differences were not statistically proven. The obtained results correspond to the notion that the leaves are varietal sign and their number is slightly affected by fertilizing. A good effect is obtained from biological fertilizing on the product part. The largest root crops, both in diameter and according to mass, were obtained after fertilizing with Italpolina and Biosol, and the differences were mathematically proven. In the other variety Large Reds results are similar. (Table 2). The mass of the plants is the largest when fertilized with Italpolina, as the difference compared to the control and it was proven at GD5%. The number of leaves was also slightly affected by fertilization, but the differences compared to the control were not proven. Very good results were found for the average mass and diameter of the root crop. The largest root crops were obtained after fertilizing with Vita organic and the difference was proven at GD 1%.

With manual scattered sowing, lower values are obtained for most of the studied indicators.

The Saxa variety forms plants with a smaller average mass compared to row sowing. (Table 3)

Table 1. Biometric characteristics of the Saxa variety under row sowing by year and average for the study period

Option	**Weight of 1 plant, g**			**Average root mass, g**			**Number of leaves per plant**			**Root diameter, mm**		
	2018-19	**2019-20**	**Average**	**2018-19**	**2019-20**	**Average**	**2018-19**	**2019-20**	**Average**	**2018-19**	**2019-20**	**Average**
K	30.11	35.45	**32.78**	12.39	16.64	**14.51**	10.12	9.98	**10.05**	31.64	27.14	**29.39**
1	31.31	36.22	**33.76**	13.50	18.44	**15.97**	10.63	10.22	**10.42**	33.27	27.66	**30.46***
2	34.68	39.15	**36.91**	13.11	19.61	**16.36**	10.13	9.96	**10.04**	33.80	27.07	**30.43***
3	30.30	36.35	**33.32**	11.67	16.66	**14.16**	10.39	10.02	**10.20**	31.96	26.67	**29.31**
	GD	5%	2.27	GD	5%	1.46	GD	5%	0.49	GD	5%	1.04
	GD	1%	4.29	GD	1%	2.40	GD	1%	0.68	GD	1%	1.51
	GD	0.1%	6.08	GD	0.1%	3.70	GD	0.1%	0.94	GD	0.1%	2.08

Table 2. Biometric characteristics of the variety Large reds in regular sowing by year and average for the period of the study

Option	**Weight of 1 plant, g**			**Average root mass, g**			**Number of leaves per plant**			**Root diameter, mm**		
	2018-19	**2019-20**	**Average**	**2018-19**	**2019-20**	**Average**	**2018-19**	**2019-20**	**Average**	**2018-19**	**2019-20**	**Average**
K	29.73	35.45	**32.59**	14.93	16.05	**15.49**	9.58	9.70	**9.64**	30.59	29.46	**30.02**
1	29.72	37.29	**33.50**	15.83	16.06	**15.94**	9.29	10.00	**9.64**	30.99	29.36	**30.17**
2	29.03	40.54	**34.78**	17.24	16.70	**16.97**	9.39	10.24	**9.81**	30.88	29.42	**30.15**
3	27.67	40.07	**33.87**	16.51	17.77	**17.14**	9.57	9.98	**9.77**	31.43	30.33	**30.88**
	GD	5%	2.18	GD	5%	1.42	GD	5%	0.40	GD	5%	1.36
	GD	1%	4.81	GD	1%	2.38	GD	1%	0.56	GD	1%	2.26
	GD	0.1%	7.65	GD	0.1%	3.28	GD	0.1%	0.77	GD	0.1%	3.51

Table 3. Biometric characteristics of Saxa variety in scattered sowing by year and average for the study period

Option	Weight of 1 plant, g			Average root mass, g			Number of leaves per plant			Root diameter mm		
	2018-19	2019-20	Average	2018-19	2019-20	Average	2018-19	2019-20	Average	2018-19	2019-20	Average
K	28.31	27.44	27.88	11.70	13.09	12.39	9.68	9.38	9.53	26.17	27.13	26.65
1	29.71	31.07	30.39	12.51	13.29	12.90	9.82	9.95	9.89	26.90	27.78	27.34
2	30.15	32.57	31.33	12.47	13.74	13.10	9.74	9.91	9.83	26.84	28.96	27.90
3	28.31	29.44	28.87	12.68	14.61	13.64	9.77	9.78	9.78	27.20	27.89	27.55
	GD	5%	2.35	GD	5%	1.11	GD	5%	0.50	GD	5%	1.36
	GD	1%	4.02	GD	1%	1.53	GD	1%	0.70	GD	1%	1.89
	GD	0.1%	7.32	GD	0.1%	2.11	GD	0.1%	0.96	GD	0.1%	2.61

The plants are the largest after fertilization with Italpolina and the difference compared to the control is statistically proven at GD 5%.

Fertilization had little effect on the number of leaves and the slight superiority of the fertilized variants was not mathematically proven. The applied biological fertilizers stimulated the formation of root crops with greater mass and diameter. The differences have been proven when fertilizing with Italpolina and Vita organic. The Large Reds variety has slightly higher values for most of the monitored indicators. (Table 4) Larger plants were formed, with the highest value when fertilized with Italpolina and the difference compared to the control was proven at GD 1%. The leaves were slightly affected by fertilization and the differences in favor of the variants with biofertilizers were not proven. This variety formed slightly larger root crops compared to the " Saxa " variety. The highest average mass and diameter have the root crops when fertilized with Italpolina and Vita organic and the differences are mathematically proven at GD 5%.

The conducted biometric research gives reason to make the following important findings: Method of sowing has an impact on the morphological characteristics. Row sowing shows an advantage over manual sowing and creates an opportunity for the formation of larger plants and better quality root crops. Biological fertilization has a stimulating effect on the morphological characteristics indicators in both varieties. The best values are obtained when fertilizing with Italpolina and the differences are proven. The stimulating effect is more pronounced with row sowing. The studied varieties respond well to the applied biofertilizers and can be recommended for use in organic production.

Good economic results when growing radishes depend not only on the development of strong plants and quality fruits, but also on the obtained ties per unit area. Yield expressed in terms of ties per hectare was reported for both methods of sowing.

In case of scattered sowing, the highest yield of "Large Reds" by year and on average for the period was reported for the version fertilized with Italpolina 8718 bunch/ha^{-1} and exceeded the control by 23.7%, and the difference was proven at GD 0.1%. Variants fertilized with Vita organic and Biosol also exceeded the control by 12% and 14% with statistical evidence at GD 0.1%. The variety " Saxa " reacted less well to fertilization with the tested fertilizers. The most connections were obtained after fertilization with Italpolina 6918 bunch/ha^{-1}. All variants with biological fertilizers exceeded the control by 7.28% to 13.19% with evidence of differences.

Yields in row sowing are higher than in scattered sowing with 11.3 % (tab. 6). When fertilizing with Italpolina, the "Large Reds" variety has the most ties by year and on average - 9950 bunch/ha^{-1}.. It surpasses the control by 24.9% and the difference has been proven at GD 0.1%. The other variants also exceed the control by 15.89% and 19.89% with a high degree of proof of the differences. The variant with Italpolina is also the most productive with the variety " Saxa " - 8096 bunch/ha^{-1}. and an excess compared to the control 16.54%. Fertilization with the other two fertilizers also helped to harvest more vines by 9.47% and 13.59% respectively over the control, with differences in all evidenced at GD 0.1%

Yield expressed in connections is strongly influenced by the method of sowing. Regular sowing creates good conditions for harvesting more summer/dec. compared to scattered hand seeding. With Large Reds, the connections in row sowing are 917 bunch/ha^{-1} (13%) more than in scattered sowing. The Saxa variety also responded well to row sowing and formed 835 more ties. Fertilization with biofertilizers further contributes to the formation of more ties by creating good conditions for germination, growth and development of seeds and plants. The sowing methods also have a significant impact on the resulting ties seed qualities.

Radishes, as a crop with a product part that is formed in the soil, are strongly influenced by the nutritional regime and are prone to nitrate accumulation. In this regard, we determined the accumulated free nitrates in the individual variants. In row sowing, no significant difference in the accumulation of nitrates was found between the two tested varieties.

Variants fertilized with organic fertilizers have higher values from 1177.4 to 1348.1 for " Saxa " and from 1023.7 to 1131.4 for "Large Reds" compared to the unfertilized control. There is no significant difference in the accumulation of nitrates when radishes are grown with scattered sowing. The highest values are when fertilizing "Saxa" with Italpolinna -1407.3. In the "Large reds" variety, fertilization with Italpolina also has higher nitrates 1051.8 compared to the control and the other fertilizers. The measured nitrates in all fertilization options are below the MPQ(maximum permissible quantities) for radishes.

The high values in the "Saxa" variety compared to "Large Reds" can be defined as a varietal reaction. The highest values reported after applying Italpolina can be explained by the chicken origin of the fertilizer. The low nitrates obtained in the row sowing can be explained by the most developed plants and the obtained more ties per unit area.

Table 4. Biometric characteristics of the variety Large reds in scattered sowing by year and average for the period of the study

Option	Weight of 1 plant, g			Average root mass, g			Number of leaves per plant			Root diameter mm		
	2018-19	2019-20	Average	2018-19	2019-20	Average	2018-19	2019-20	Average	2018-19	2019-20	Average
К	29.96	30.86	30.41	12.94	13.14	13.04	9.95	10.18	10.07	26.81	28.30	27.55
1	34.01	31.36	32.68	13.10	14.17	13.64	10.25	10.64	10.45	27.35	29.71	28.53
2	35.56	36.06	35.81	13.08	14.87	13.98	10.25	10.46	10.36	27.88	29.49	28.80
3	30.90	34.75	32.85	13.90	14.47	14.19	10.30	10.05	10.18	27.99	29.10	28.55
	GD	5%	2.47	GD	5%	1.06	GD	5%	0.39	GD	5%	1.24
	GD	1%	4.59	GD	1%	2.40	GD	1%	0.62	GD	1%	1.91
	GD	0.1%	6.78	GD	0.1%	3.70	GD	0.1%	1.04	GD	0.1%	2.78

Table 5. Yield with scattered sowing by year and average for the study period - pieces/ha^{-1}

Option	Large reds				Saxa			
	2018-19	2019-20	Average	%	2018-19	2019-20	Average	%
К	6575	7523	7049.0	100	5826	6398	6112.0	100
1	7447	8466	7956.5	112.87	6318	6795	6556,5	107.27
2	8129	9307	8718.0	123.67	6621	7216	6918.5	113.19
3	7674	8443	8058.5	114.32	6508	7250	6879.0	112.54
	GD	5%	540				346	
	GD	1%	702				561	
	GD	0.1%	935				752	

Table 6. Yield with regular sowing by year and average for the period of the study - pieces/ha^{-1}

Option	Large reds				Saxa			
	2018-19	2019-20	Average	%	2018-19	2019-20	Average	%
К	7740	8193	7966.5	100	6405	7489	6947.0	100
1	8626	9840	9233.0	115.89	7249	7961	7605.0	109.47
2	9478	10423	9950.0	124.91	7738	8454	8096.0	116.54
3	9163	9940	9551.5	119.89	7591	8191	7891.0	113.59
	GD	5%	623				401	
	GD	1%	836				585	
	GD	0.1%	989				803	

Table 7. Nitrate content in root crops in row sowing - mg/kg

Option	**Saxa**				**Large reds**			
	2018-19	%	2019-20	%	2018-19	%	2019-20	%
К	1218.6	100	1087.4	100	951.7	100	891.3	100
1	1375.3	112.9	1177.6	108.3	1078.5	113.3	969.7	108.8
2	1581.9	129.8	1348.1	123.9	1223.9	128.6	1051.8	118.1
3	1418.1	1116.4	1299.3	119.5	1201.5	126.2	1003.5	112.6

Table 8. Nitrate content in root crops with scattered sowing - mg/kg

Option	Saxa				Large reds			
	2018-19	%	2019-20	%	2018-19	%	2019-20	%
К	1183.5	100	997.1	100	993.1	100	918.5	100
1	1351.7	114.2	1118.4	117.5	1118.3	112.6	1023.7	111.5
2	1615.3	136.5	1407.3	141.1	1293.7	130.2	1131.4	123.2
3	1458.1	123.2	1253.6	125.7	1201.5	119.3	1105.3	120.3

CONCLUSIONS

Fertilization with the organic fertilizers Italpolina, Vita organic and Biosol has a stimulating effect on the morphological indicators and the quality of the root crops in Saxa and Large Reds" . Both varieties can be successfully used in organic production in greenhouse conditions. Row sowing has an advantage over scattered sowing and creates conditions for the formation of better quality root crops and obtaining more bunches per ha$^{-1.}$ The organic fertilizers used do not cause accumulation of nitrates in root crops above maximum permissible amounts of nitrates.(MPA)

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DROUGHT STRESS AND THE ROLE OF SALICYLIC ACID IN RELIEVING THE OXIDATIVE DAMAGE AT TOMATO PLANTS

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Abstract

Our study was accomplished in lab conditions at Vegetables Research and Development Station Bacău, aiming to determine the impact of foliar treatment with salicylic acid (SA) over the ability of plants to overcame the water stress. The pot experiment used a randomized block design with four replications and ten plants per variant. Leaves from well-developed plants (30 days old seedlings) were sprayed with 1 mM SA solution until both sides were completely moistened. After treatment application, water regime followed as one set was used as a well-water treatment (WW) and the other set as a drought stress treatment (DS) where the plants were restricted watering for the next 10 days. Results showed that tomato plants treated with salicylic acid and exposed to water stress become more tolerant to drought stress induced oxidative damage than those not treated, the values were comparable to those recorded in WW plants. The extent of water stress injury was higher at non-treated DS plants. The results obtained showed that foliar application of SA stimulated the adaptation of plants, improving water-plant relation, allowing to alleviate the oxidative damage.

Key words*: plant growth, stimulating growth, stress factors, VRDS Bacău.*

INTRODUCTION

The climate is changing faster than some cultivated plant species can adapt, raising uncertainties about the future performance of cultivated vegetables (Lippmann et al., 2019; Pironon et al., 2019; Zsögön et al., 2022). In Romania, results from climate studies show a general trend toward warmer and drier conditions over the last 30 years, with the average annual temperature increasing by 0.3-1.1°C (Mihai et al., 2022).

Stressors such as salinity, drought, temperature extremes, and nutrient deficiencies can have an adverse effect on plants (Mimouni et al., 2016; Kul et al., 2020). Water stress is by far the most important environmental constraint in agriculture (Muñoz-Espinoza et al., 2015), which slows plant growth and development and reduces crop production (Fan et al., 2022). Many factors contribute to a water deficit in plant, and these include among others low rainfall, soil salinity, high and low temperatures and high light intensity. Drought stress often affects plants in both natural and agricultural environments (Salehi-Lisar & Bakhshayeshan-Agdam, 2016; Ojuederie et al., 2019; Seleiman et al., 2021). To reduce the negative effects of drought on their physiology, plants have evolved a number of strategies and a highly coordinated hormonal network that are crucial for this process (Muñoz-Espinoza et al., 2015; Wahab et al., 2022).

Salicylic acid (SA) is produced by many organisms, including plants, and is a water-soluble secondary metabolite and phenolic compound (Souri & Tohidloo, 2019). All plants contain SA which is part of the group of plant hormones (Yusuf et al., 2013).

Several studies have shown that SA, if is applied exogenously, in low doses, increases endogenous levels and improves plant growth and development and can play a key role in relieving as well as reducing plant stress (Dempsey et al., 2011; Lakzayi et al., 2014; Wani et al., 2017; Lefevere et al., 2020; Mardani-Mehrabad et al., 2020; Sharma et al., 2020; Chakma et al., 2021; Aires et al., 2022). Also, foliar applied SA improves photosynthesis, growth, and various other physiological and biochemical features in stressed plants (Wani et al., 2017).

Using biostimulants such as SA is an emerging novel practice to improve crop yield and quality because it regulates various physiological and metabolic processes (Nephali et al., 2020; Sariñana-Aldaco et al., 2020). There is no doubt that plants respond to SA in different ways, but how they respond depends on the cultivar, the environment, and the concentration of SA (Rivas-San Vicente & Plasencia, 2011; Orabi et al., 2015; Kumaraswamy et al., 2019).

Tomato (*Lycopersicum esculentum* Mill.) is one of the vegetables that has recently been added to the list of the world's most important food crops (Endalew, 2020; Shafiwu, 2021). It is an herbaceous annual plant from Solanaceae family grown for its edible fruit. The tomato plant produces yellow flowers, and usually a round fruit, which can be red, pink, purple, brown, orange or yellow (Bergougnoux, 2014; Ho, 2017; Quinet et al., 2019). Romania has a high potential export market for this fruit (Tomescu & Negru, 2002; Nour et al., 2013; Soare et al., 2017;).

Worldwide, drought stress severely limited the tomato crop production (Liu et al., 2021). The drought tolerance of plants can be improved. Is demonstrated that the application of SA can reduce the harmful effects of environmental stress on tomato (Aires et al., 2022).

SA is considered an endogenous regulator, and its role in the mechanisms of defence against biotic and abiotic stress has been documented (Javaheri et al. 2012; Mohamed et al., 2020), but, in tomatoes case, the plants are particularly sensitive to SA treatments (Sariñana-Aldaco et al., 2020). Therefore, the aim of this study was to evaluate the effect of foliar treatment at *L. esculentum*, Bacuni variety, with 1 mM SA on growth characteristics of tomato seedlings under drought stress.

MATERIALS AND METHODS

The experiment was carried out in 2022, at Vegetables Research and Development Station from Bacău in a growth chamber.

Plant material and growth conditions

The seeds of *Lycopersicon esculentum* L. Bacuni variety, was purchased from Bacău Vegetables Research and Development Station. The tomatoes seedlings were uprooted from the germinal bed and transplanted in pots with a 7.8 cm diameter and a volume of 150 mL. The plants were maintained in growth chambers, at an average temperature of 25°C ± 2°C. The soil substrate was represented by peat from NAVARBO with a nutrient content of soluble nitrogen (N) - 163 mg/L, nitrate nitrogen (NO_3-n) - 98 mg/L, Ammonium-N (NH_4-N) - 65 mg/L, soluble phosphorus - P (P_2O_5) - 195 mg/L, soluble potassium - K (K_2O) - 217 mg/L + microelements [(boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn)], with a potential of hydrogen (pH) - 5.5, electrical conductivity (EC) = 31 mS/m (1: 5). The peat granulation was between 0-10 mm. All plants were watered every two days for 30 days after planting the seedlings in pots. On the 30th day after transplanting, the plants were divided into different groups, which we called variants, and treated with 1 mM concentration of SA. The first variant, well water (WW), was the control variant. No treatment with SA was applied and the plants were watered regularly (every 2 days). The second variant was regularly watered and treated with salicylic acid (WW+SA). The stress was imposed by stopping the watering of the plants for a period of ten days after the foliar treatment with SA. Therefore, plants from the third variant were under drought stress (DS) conditions and no foliar SA treatment was applied. In the fourth variant, plants were placed under drought stress conditions and treated with salicylic acid (DS+SA). All plants on the experimental variants were watered on day 11 after foliar treatment application. Plant samples were collected 48 hours after the last watering to evaluate the parameters listed below.

Wilting degree

The leaf wilting index of tomato plants was performed according to the wilting scale established by Mai-Kodoni (MAIK), described by Alidu et al. (2019). In our experiment, the wilting index was determined on the 11th day after the application of the foliar treatment on tomato plants.

Plant measurements

All plants in the experimental variants were watered after collecting wilting index data. After 48 h, biometric measurements were performed: plant height (mm), shoot length (mm), root length (mm), wet weights of shoots and roots (g) and leaf surface represented by leaf area (mm^2), leaf length (mm), leaf width (mm), and the ratio factor between length and width.

Leaf area was measured with the ADC AM350 portable leaf area meter.

RESULTS AND DISCUSSIONS

The results showed that drought stress conditions reduced the growth of tomato seedlings. The foliar application of SA also increased the fresh and dry weight of the roots.

To breed plants with greater stress tolerance, the tomato leaf wilting index is used as a visual indicator of low moisture stress (Pungulani et al., 2013). As shown in Figure 1, the plants most affected by water stress were the DS plants with a wilting index of 70%.

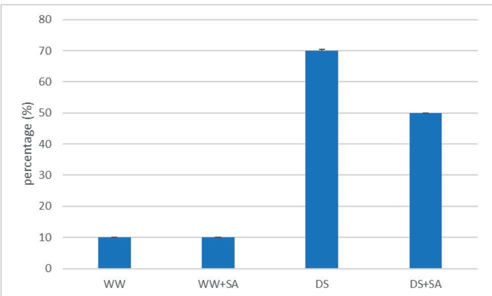

Figure 1. Graphical representation of tomato leaf wilting index means values in day 11 from after SA foliar treatment application

Tomato plants on DS+SA had a wilting index of 50%, which supports the conclusion that treatment with 1 mM SA enhance the resistance of plants to water stress with 20% compared with DS. As a result, the wilting process of drought-stressed plants is obviously slowed down in the DS+SA variant.

Tomato plants showed differences in growth and development according to the experimental variants studied. The SA treatment supported plant growth and development processes under drought stress conditions. The length of plants (Figure 2, blue color) recorded on the DS+SA variant was comparable to those recorded on the control variant (WW).

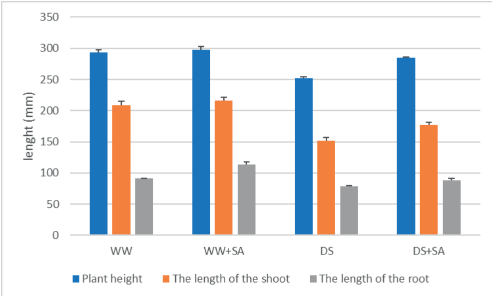

Figure 2. Graphical representation of plant height, shoot length and root length 13 days after SA foliar treatment application

Also, the plants from WW+SA variant recorded higher biometric values compared to the WW control variant. The length of shoots and roots plants from DS+SA variant was higher than plants from DS variant (Figure 2, orange and grey color). We also observe a more uniform growth of plants on the DS+SA variant compared to the other plants grown on studied variants.

Exogenous application of SA has been shown to improve plant growth and development in several studies (Dempsey et al., 2011; Lakzayi et al., 2014; Wani et al., 2017; Lefevere et al., 2020).

In our study the foliar application of 1 mM SA treatment stimulated the growth of tomato plants in both WW+SA and DS+SA variants compared the variants without SA. These results are supported by those of Javaheri et al. (2012) who studied the effects of SA on yield and quality characters of tomato fruit. Foliar application of salicylic acid improves the quantity and quality of tomato fruits (Javaheri et al., 2012).

Also, the foliar application of SA increased shoot and root fresh weight compared to DS and WW. As shown in Figure 3 (blue color), the WW+SA variant has 8.25 g, with a difference of 0.75 g compared to the WW variant. The DS+SA variant has a 7.37 g difference of 0.62 g compared to the DS variant. When it comes to roots (Figure 3, orange color), the differences are smaller, but the mass is higher for variants that have been treated with SA than the variants without SA treatment.

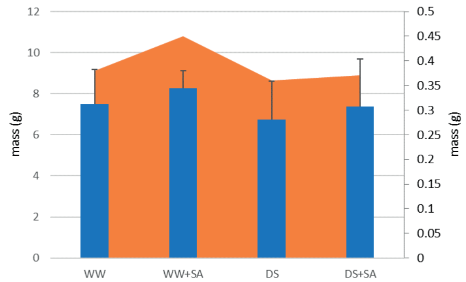

Figure 3. Graphical representation of wet mass of shoots and roots in the experimental variants, 13 days after SA foliar treatment application

Orabi et al. (2015) say that 0.5 mM and 1.0 mM SA treatments have a positive significant effect on growth of tomato plants under low temperature conditions in sandponic culture.

Many studies have shown that the negative impact of heat and water stress can be reduce through the application of SA (Lefevere et al., 2020; Mardani-Mehrabad et al., 2020; Sharma et al., 2020; Chakma et al., 2021; Aires et al., 2022). That's why, foliar surface, specifically LA is a crucial index, indispensable for many physiological models, because LA indicates the rate of photosynthesis, with impact on stress resistance. The results obtained in terms of LA ratio accurately express the relative growth rate changes determined by the stress factors studied. As shown in Figure 4, WW+SA treated variants have the highest LA, leaf length, and leaf width. A higher LA is observed for the DS+SA variant plants compared to the plants from DS variant. Physiological activities of tomatoes are affected by salicylic acid application, which highlights its role in regulating drought stress responses (Javaheri et al. 2012, Chakma et al., 2021, Liu et al, 2021, Fan et al., 2022).

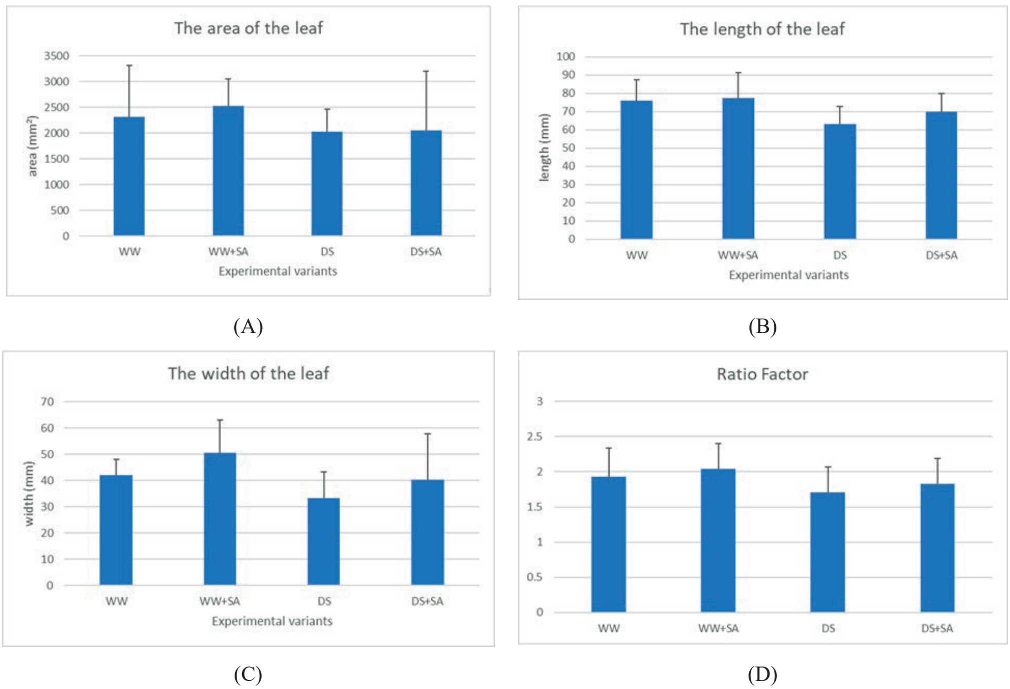

Figure 4. Graphical representation of tomato leaves growth parameters

CONCLUSIONS

Tomato plants wilted quickly and grew poorly in drought circumstances. Foliar application of 1mM salicylic acid (SA) treatment increased tomato seedling development in WW+SA and DS+SA variations when compared to untreated variants. Significant effects were observed on shoot and root length, shoot and root fresh weight, and leaf area. Comparatively to the DS variant, the foliar application of SA at a concentration of 1 mM in the DS+SA variant improved seedling growth. The effects of SA acid treatment on WW+SA variant on tomato seedling is higher than those of the WW control. The foliar application of SA has the potential to control the growth and development of plants cultivated in greenhouses and field, enabling the activation of the plant's response to water stress challenges.

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VEGETABLES CROPS NUTRITION MANAGEMENT USING DRIP FERTIGATION AND SHADING MESH

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Abstract

The main objective of our case study was related to a comparative assessment of chemical and organic fertilizers on yield and quality of vegetables in arid conditions. The highest yield of two pepper hybrids was fixed in the trial fertigated with liquid digestate fraction and equipped with sunlight protective mesh.
The fresh red tomato yield was highest after foliar spraying treatment with vermicompost tea (6.95 kg/m^2) and carbamide (7.23 kg/m^2) in the second field experiment. The relative additional yield of aubergine fruits (11.6-12.1%) and pepper (12.0-12.7%) was higher in case of drip fertigation. The aubergine fruits have a high peroxidase activity. The high activity of peroxidase was especially observed in fruits when using vermicompost tea. The use of drip fertigation proved to be a more efficient operation of the peroxidase system in pepper fruits compared to foliar spraying. The study of molecular forms of peroxidase in tomato seeds showed their small number (six components) and variability of expression under different growing conditions. Significant inhibition of isoperoxidase activity was observed in carbamide treatment after foliar spraying of tomato plants.

***Key words**: digestate, drip fertigation, shading mesh, vegetables crops, vermicomposting tea.*

INTRODUCTION

Pre-conditions to organic farming of vegetables growing. During last decades agriculture in the steppe zone of Ukraine is in most cases caused by changing weather conditions and the diversity of soil fertilizers cover. Precipitation is less than 350 mm in dry years i.e. dry steppe zone, in damp years more 550mm in forest-steppe zone (average perennial value 470 mm).

Recently, Ukrainian private farms have used very little fertilizer. Generally speaking, farms have been forced into this situation because they do not have enough money to buy expensive mineral fertilizers.

Vast quantities of nutrients exist in agri-wastes that can potentially be utilized as fertilizers or soil amendments to subsidies the tremendous demand for synthetic chemical fertilizers and to reduce the economic and environmental costs associated with fertilizer production and waste disposal.

Soild and liquid fractions of digestate prospects. The market value of digestate products is usually estimated at the equivalent market value of nutrient analogues (N, P, and K) in mineral fertilizers. Therefore, both solid and liquid fractions of bio-digestate are prospect products to use it following famous agricultural practices (Tambone et al., 2017; Logan & Visvanathan, 2019). As a rule, biogas plants use a mixture of different types of raw materials. The results of a study conducted by KTBL (KTBL, 2008) showed that any further processing of 'raw' digestate, regardless of the type of derivatives thereof, leads to a decrease in the equivalent net value of digestate by the NPK complex, and therefore for most biogas plants simple soil application of digestate is more economically attractive than digestate processing. The ultrafiltered dairy manure digestate biofertilizer had the highest yield of red tomatoes (7.13 tons/ha) followed by the concentrated food waste digestate biofertilizer and mineral N fertilizer treatments with 6.26

and 5.98 tons/ha, respectively. The digestate provided a significant amount of ammonium N, which is rapidly nitrified and therefore available directly to crops in the short term (Alburquerque et al., 2012). Furthermore, the addition of digestate led to an increase in the amount of P available in the soil. The subsurface fertigation of digestate can be useful in decreasing the volatilization of ammonia in fields after digestate application (Lili et al., 2016). Meanwhile, this liquid digestate application technology may be a cost-effective option compared to chipper surface drip fertigation (Nkoa, 2014). In this sense, the use of poultry digestate liquid fraction for irrigation and fertigation appears very promising as well. However, attention must be paid to avoid overapplication of digestate in the soil, including several negative environmental effects including leaching, phytotoxicity, soil salinity, pathogen exposure, and increased gaseous NH_3 emissions (Barzee et al., 2019).

Vermicomposting as main component of organic farming in horticulture. It is known that vermicomposting is the product of accelerated biodegradation of organic matter by earthworms through mesophilic decomposition (Márquez-Quiroz et al., 2014). Vermicomposts are finely divided peat-like materials with high porosity, aeration, drainage, water-holding capacity, and microbial activities, which make them excellent soil amendments or conditioners (Atiyeh et al., 2000). These composts result from a non-thermophilic biodegradation and stabilization of organic materials because of earthworms and microorganisms' interactions (Agnieszka et al., 2013). The composition of vermicompost-based growth substrates includes 25-30% vermicompost, peat, sand, gravel and perlite (Olle, 2016). During the mean time certain proportions of vermicompost and natural soil from 1: 1 to 4: 1 can be prepared as well (Abduli et al., 2013). Four levels of vermicompost (0, 5, 10 and 15 tons/ha) were applied in the field experiment as an easy bio-treatment to improve pepper antioxidant compounds, fruit quality and yield (Aminifard & Hassan, 2016). Vermicompost provided pepper plants with the highest growth rate during the nursery period, especially under salinity stress conditions (Kaciu et al., 2011). Vermicompost and chicken manure compost more effectively promoted tomato plant growth, including stem diameter and plant height compared to other fertilizer treatments, in all three types of soil (Wang et al., 2017). The rate 0.5-0.6 g/g of vermicompost added to soil provided tomato growth similar to the standard inorganic fertility program (Zucco et al., 2015). The production of solanaceous crops can be improved also with vermi-tea fertigation (Aslam et al., 2020).

Vermicomposting tea is a mixture of aerobic microbes present in vermicomposting with aerated water (Arosha & Sarvananda, 2022). Incorporation of aerated compost tea obtained from organic compost based on the mixture of rice straw compost, vermicompost, and Hinoki cypress bark compost in the root zone increased shoot and root growths and yield of red leaf lettuce (Kim et al., 2015). Vermicompost tea can be irrigated, sprayed plants or sprinkled on leaves, as it contains many useful nutrients, growth regulators, and enzymes (Alkobaisy et al., 2021). Low concentrations of vermi-compost tea at rates of 1.6% and 3.2% for lettuce significantly increased lettuce yields (Arancon et al., 2019). Even lower concen-trations of vermicompost tea significantly increased tomato yields as a supplement in reduced nutrient solutions of 50%.

Isoperoxidase activity as index of fruits quality. It is known that such enzyme as isoperoxidaze may influence the quality of fruits and vegetables (Muftugil, 1985). Products with high peroxidase activity must be blanched or treated with antioxidants to decrease isozyme activity (Prestamo & Manzano, 1993). Those vegetables that exhibit low peroxidase activity and mainly contain low-molecular-weight isozymes need not be blanched, but can be treated with antioxidants if needed.

The main objective of our research was related with effect of liquid fraction of digestate, carbamide and vermi-tea on yield and quality of tomato, pepper and aubergine fruits in arid conditions.

MATERIALS AND METHODS

Site location and soil conditions.This research work was carried out at the Pokrov research and educational station located in the southern part of Ukraine (47º39'N, 34º08'E). The annual rainfall and evaporation are 465 mm and 650 mm accordingly. The black soil used for the experiment has loess like loam texture.

Experimental design. The layout of the two field experiments was designed in a completely randomized design with three replicates at 2018 and 2021.

First field experiment. Two pepper varieties (Monolite and Turmaline) were selected to examine the effectiveness of different trials: **a)** control (water); **b)** treatment with drip fertigation using the liquid fraction of digestate (LFD) dissolved by water in ratio 1:200; **c)** with and without sun protective mesh (SPM) and **d)** LFD+SPM (Figure 1).

Figure 1. Pepper drip fertigation under sun protective mesh

Fertilization in first field experiment. Poultry litter digestate was obtained from MHP Oril-Leader farm involved with all aspects of poultry agriculture. In 2012, it commissioned an anaerobic digester in Yelyzavetivka village for poultry manure and slaughterhouse waste using technology from Nijhuis Industries.

Three liquid fraction of poultry litter digestate injection with drip irrigation system were made during vegetation period. The protective mesh was applied as a second test with the intention of reducing the impact of exposure to sunlight on the volatilization of ammonia.

Second field experiment. Three varieties of tomato (Bobkat), aubergine (Nadir) and pepper (Ayvengo) were tested to compare foliar spraying and drip fertigation.

The treatments were: (**1**) no fertilizer (water), (**2**) mineral N fertilizer (carbamide), (**3**) vermicompost tea (vermi-tea). The dose of 20 kg of carbamide was dissolved in 10 litres of water. The vermicompost tea was dissolved by water in a 1: 100 using an injector (Figure 2).

Figure 2. Fertilizer delivery with injection system

The fertilization rate was taken following recommendations for artificial ammonium liquid fertilizers to obtain optimal growth of drip irrigation vegetables in Dnipropetrovsk province (Kharytonov et al., 2019).

Fruit determinations. The mass of the vegetable fruits was recorded for each plot. For the determination of the dry vegetable yield, five of the fruits of each treatment plot were randomly sampled, cut, and weighted in kg/m^2. The nitrogen content in fruits was analyzed with the Kjeldahl method. The fruits of pepper,aubergine fruits and tomato seeds (0.3 g) were homogenized in 6 mL of 0.05 M Tris-HCI buffer, pH 7.4 with 0.5% polyvinylpyrrolidone (PVP) for the isolation of the benzidine peroxidase. The extraction was carried out at +40^{0}C for 1 hour. The extract was centrifuged for 15 min at 14,000 rpm. The supernatant was selected to determine the isoenzyme composition of benzidine peroxidase (BPx). The isoenzyme composition of BPx was determined by the isoelectric focus (IEF) method on a 5% horizontal polyacrylamide gel (PAAG) on an "Ultrophor" device (LKB, Bromma, Sweden), pH range 3.5-6.5. The benzidine method was used to detect enzymatic activity in a polyacrylamide gel (Guikema & Shermen, 1980).

Data analyses. The data obtained were processed using statistical methods using the StatGraphics Plus software package at a significance level of 0.95 % (P-value < 0.05).

RESULTS AND DISCUSSIONS

Pepper fruits yield and quality in experiment with liquid fraction of digestate
The highest yield of two pepper hybrids was fixed in the LFD + SPM trial fertigated with liquid digestate fraction and equipped with sunlight protective mesh (Figure 3).

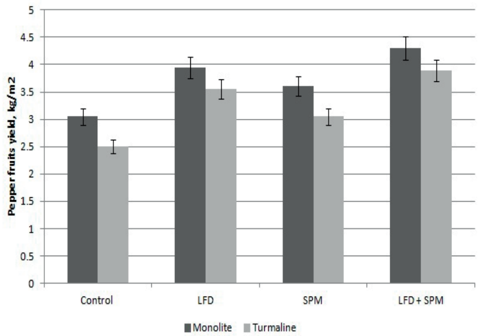


Figure 3. Pepper fruits yield, kg/m^2

In the meantime, the lowest data on dry matter in pepper fruits was fixed in the LFD + SPM trial as well (Figure 4).

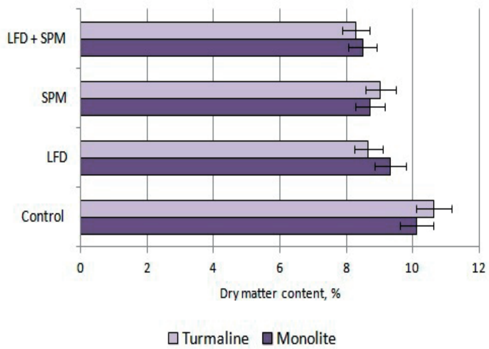


Figure 4. Dry matter content, %

The data on nitrogen content in pepper fruits is shown in Figure 5. The maximum level of nitrogen content in Monolite (1.85%) and Turmaline (2.13%) pepper hybrids fruits was found after drip fertigation with liquid fraction of poultry litter digestate.

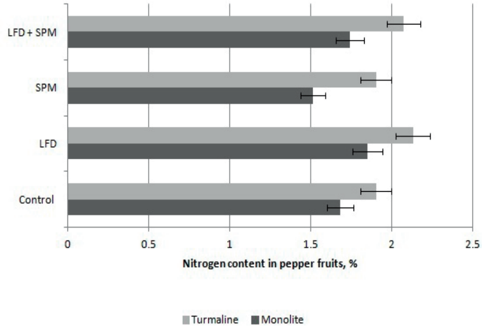


Figure 5. Nitrogen content in pepper fruits, %

Comparative presentation of tomato, pepper and aubergine fruits yield
The yield data obtained in the field experiments with tomato, aubergine and pepper are shown in the Figure 6 and Figure 7. The fresh red tomato yield was highest after foliar spraying treatment with vermicompost tea (6.95 kg/m^2) and carbamide (7.23 kg/m^2).

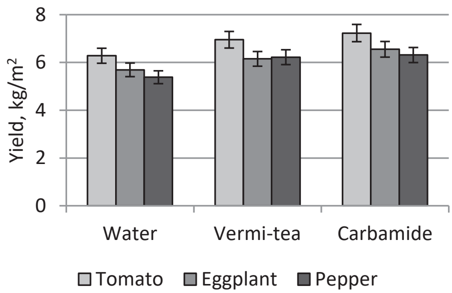


Figure 6. Foliar spraying fertilization effect on vegetables yield

The relative additional yield of aubergine (11.6-12.1%) and pepper (12.0-12.7%) was higher in the case of drip fertigation (Figure 7).

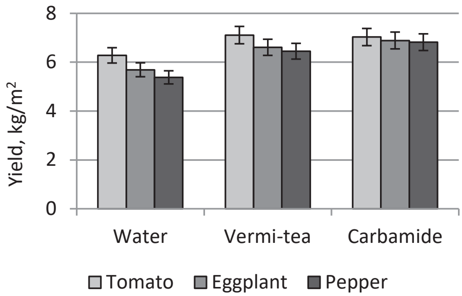
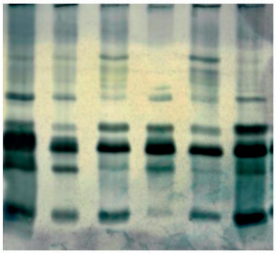


Figure 7. Drip fertigation effect on vegetables yield

Meantime, the tomato drip fertigation with vermicompost tea provided maximum yield (7.11 kg/m^2) or 11.3% comparative to control.

Drip fertigation and foliar spraying impact on tomato, pepper and aubergine enzymatic antioxidant system.

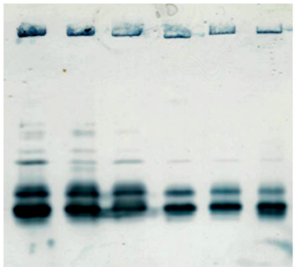
The study of the expression of redox enzymes occupies a special place in the evaluation of the adaptation capabilities of various plant tissues. These enzymes directly or indirectly affect the concentration of reactive oxygen species in tissues (Clemens, 2001; Mittler et al., 2004). It was shown that aubergine fruits have a high peroxidase activity. The structure of the aubergine fruits IEF spectra of peroxidase was similar both under drip irrigation and under foliar spraying (Figure 8).



1 2 3 4 5 6

Figure 8. Changes in the polyacrylamide gel isoelectric focusing (IEF) profiles of benzidine-peroxidase from aubergine fruits are grown using two methods of plant treatment with different types of fertilizers: Drip irrigation: 1 - Vermi-tea; 2 - Water; 3 - Carbamide; Foliar spraying: 4 -Water; 5 - Carbamide; 6 - Vermi-tea

The highest activity was found for four enzyme isoforms with pI 4.00, 4.12, 4.20 and 4.30. Especially high activity of peroxidase was registered in fruits when using vermicompost tea. Isoperoxidase with a pI of 4.20 showed consistently high activity in all treatments. Significant fluctuations were observed in the activity of components with a pI of 4.00, 4.12 and 4.30. Isoperoxidase with a pI of 4.00 showed the highest activity in the treatment with vermi-tea, and the lowest - after foliar spraying with water. The component with pI 4.12 showed high activity after foliar spraying with vermi-tea. Treatment with carbamide leads to inhibition of this isoform. This has also been observed with drip fertigation. The highest activity of the enzyme isoform with a pH of 4.30 was recorded in the vermi-tea trial with both methods of aubergine treatment. The lowest activity of the isoenzyme was fixed in the trial with water during drip irrigation. The average intensity of the component with a pI of 4.30 was observed in treatments with carbamide after foliar spraying. Minor forms (range of pI values 4.60-5.05) of peroxidase also showed variability in activity in different approaches with different types of fertilizers. Research on the antioxidant activity of pepper fruits was also carried out based on the study of the isoenzyme composition of benzidine peroxidase (Figure 9). The use of drip fertigation proved a more efficient operation of the peroxidase system in pepper fruits compared to foliar spraying.



1 2 3 4 5 6

Figure 9. Changes in the polyacrylamide gel isoelectric focusing (IEF) profiles of benzidine-peroxidase from pepper fruits are grown using two methods of plant treatment with different types of fertilizers: Drip irrigation: lanes 1 - Vermi-tea; 2 - Water; 3 - Carbamide; Foliar spraying: lines 4 -Water; 5 - Carbamide; 6 - Vermi-tea

The treatment with vermi-tea stood out in particular. The study of molecular forms of peroxidase in tomato seeds showed their small number (six components) and variability of expression under different growing conditions (Table 1).

Table 1. The polyacrylamide gel isoelectric focusing (IEF) profiles of benzidine-peroxidase from tomato seeds

pI	Foliar spraying			Drip irrigation		
	vermi-tea	water	carbamide	water	carbamide	vermi-tea
4.00	+++	+	++	+	++	+++
4.10	++++	+	+	+	++	+++
4.15	+	-	-	-	-	+
4.95	+	-	-	-	-	+
5.10	trace					+
5.95	trace	trace	trace	trace	trace	trace

Components with a pH of 4.00 and 4.10 showed high activity in the vermi-tea treatment both during drip irrigation and foliar spraying. Significant inhibition of isoperoxidase activity is observed in carbamide treatment after foliar spraying of tomato plants. Therefore, the results of studying the composition of the isozyme showed significant changes in the number of molecular forms of peroxidase. The variation in its level of its expression along their distribution in the spectrum confirms the polyfunctionality of this enzyme depending on the conditions of plant growth (Shupranova et al., 2019). Such changes may be caused by post-translational modifications, increased synthesis of the enzyme, or the appearance of its new isoforms (Kumar et al., 2014). The results obtained showed a high activity of the peroxidase system with the use of vermi-tea extract in pepper and aubergine fruits. This may be related to the neutralization of active forms of oxygen formed when the intensity of respiration in ripe fruits increases. A strong correlation between respiration intensity and peroxidase activity was also shown in plum fruits (Serdyuk et al., 2017).

A change in the activity ratio of the molecular forms of peroxidase in the pepper, aubergine fruits, and tomato seeds is one of the consequences of the treatment of the plant with different types of fertilisers.

CONCLUSIONS

The main objective of our study was related to a comparative assessment of chemical and organic fertilizers on yield and quality of vegetables in arid conditions. The layout of the field experiments was designed with three replications in 2018 and 2021.The liquid fraction after liquid/solid separation of biogas digestate has a high potential as a fertilizer as a result of its high nutrient concentration. The highest yield of two pepper hybrids was observed in the trial fertigated with liquid digestate fraction and equipped with sunlight protective mesh. The maximum nitrogen content in the fruits of Monolite (1.85%) and Turmaline (2.13%) pepper hybrids was determined after drip fertigation with the liquid fraction of poultry litter digestate. The fresh red tomato yield was highest after foliar spraying treatment with vermicompost tea (6.95 kg/m^2) and carbamide (7.23 kg/m^2). The relative additional yield of aubergine (11.6-12.1%) and pepper (12.0-12.7%) was higher in the case of drip fertigation. Meanwhile, tomato drip fertigation with vermicompost tea provided maximum yield (7.11 kg/m^2) or 11.3% compared to the control.

The use of drip fertigation proved to be a more efficient operation of the peroxidase system in pepper fruits compared to foliar spraying. Significant inhibition of isoperoxidase activity is observed in carbamide treatment after foliar spraying of tomato plants.

Sunlight protective mesh is only one way to decrease ammoniac evaporation using a liquid fraction of digestate for fertigation. The incorporation of some natural adsorbents (biochar and zeolites) into the topsoil seems very promising to decrease negative consequences connected with NO_x emission, nitrates, mobile phosphates leaching, etc.

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ONION STEMPHILIOSIS IN SOUTHERN UKRAINE

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Abstract

Stemphiliosis of onion is a relatively new disease in Ukraine. The disease appears in the form of oblong spots on the leaves and causes premature leaf death. The aim of the work is to propose means of protection for control of onion stemphyliosis based on the study of the pathocenosis species composition, biophenology of the disease agent of onion stemphyliosis and determination of the fungicides efficiency. Field studies were carried out on plantings of onion variety Banco in the farm AF "Petrodolynske" Odessa district, Odessa region of Ukraine in 2019-2021. We identified the causative agents of onion stemphyliosis - Stemphylium vesicarium (Wallr.) Simm, Stemphylium botryosum Wallr, Stemphylium herbarum Simm. which had different frequency of occurrence in the samples. The preparations with the following active substances were found to control the disease most effectively under field conditions: fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + prothioconazole (100 g/l).

Key words*: onion, phytosanitary monitoring, Stemphylium vesicarium, fungicides.*

INTRODUCTION

Onion (*Allium cepa*) are one of the most important vegetable crops in the world, being the second most valuable after the tomato. Onion yields can be significantly reduced due to many factors, including disease. In recent years, there has been a widespread and increasing harmfulness of onion stemphyliosis or black-gray spot disease. S.M. Stricker et al. (2020) indicate that stemphiliosis, by causing premature leaf dieback, shortening the period of weight gain and ripening, can result in 28-38% loss of onion yield and 74% loss during epiphytotics. M. Hausbeck and B. Werling (2018) note that the disease has been sporadically reported on onion leaves for the past 30 years and was considered a secondary pathogen, but in recent years *Stemphylium vesicarium* has become the dominant pathogen, which appears to have displaced *Alternaria porri*. F. Hay (2018) attributes this to the development of resistance to commonly used fungicides.

The causative agent of Stemphyliosis is the imperfect fungus *Stemphylium vesicarium* (Wallr.) E.G. Simmons, 1969 (teleomorph: *Pleospora alii*). M.E. Miller and H.F. Schwartz (2008) reported that onion stemphiliosis occurs in many regions of the world where onions and garlic are cultivated, but it causes the most problems in warm regions. The main source of the disease is infected plant debris as well as bulbs of infected plants on which the fungus can persist for a long time (Basallote-Ureba et al., 1999). Stemphiliosis reinfestation during onion growing season is carried out by conidia. Conidia can enter onion leaves through stomata (Tayviah, 2017), damage caused by other diseases, insect pests (tobacco thrips, onion flies), herbicides, and wind and hail (Nischwitz, 2016; Du Toit L., 2017). Du Toit L. (2017) stated that onion plants that have been exposed to heat stress become more susceptible to stemphyliosis.

The first signs of the disease on the leaves and leaf sheaths appear as small watery spots that vary in color from light yellow to brown. Increasing in size, the affected areas merge, causing extensive leaf spotting. The central area of the affected areas turns brown to yellowish-brown, then dark olive-brown, and finally black when the fungus spores. Sometimes the fruiting bodies of the fungus, called pseudothecia, may form in the affected tissue as small, black, pinhead-like, raised bodies. The infection usually affects only the leaves and does not spread to the bulbs. The development of stemphiliosis is favored by temperatures between 15 and 25°C, humid conditions, and prolonged periods of leaf wetness lasting 8 hours or more (Suheri and Price, 2000). M.E. Miller and H.F. Schwartz (2008) found that under favorable conditions: temperatures above 18°C, high concentration of conidia in the air, and 24 hours of continuous wet weather, the number of conidia on the leaf surface can exceed 200 pieces per square centimeter. Sporulation usually occurs at the site of initial lesions and is observed 6-14 days after lesion development (Basallote-Ureba et al., 1999). *Stemphylium vesicarium* is generally considered a secondary pathogen that attacks previously damaged tissue; however, severe damage to healthy leaves may be observed during warm weather when leaves are wet for more than 24 hours (Miller and Schwartz, 2008).

Control of stemphyliosis can only be achieved by providing an integrated approach that includes agronomic techniques, biological, chemical and other methods. Agrotechnical methods of controlling stemphyliosis are aimed at reducing the burden of the pathogen on seeds and creating conditions less favorable for infection. Deep tillage after onion harvest promotes decomposition of plant residues and prevents airborne release of ascospores from pseudothecia (Basallote-Ureba et al., 1999; Paibomesai and Celetti, 2012).

Crop rotation with a rotation of 3-4 years reduces the amount of infection in the soil, thereby reducing the incidence of onion stemphiliosis. Cautious use of nitrogen fertilizers is recommended because excessive nitrogen application increases disease severity (Nischwitz, 2016). Du Toit (2017) stated that drip irrigation reduces the risk of stemphiliosis infection by reducing the wetting period of the crop. Other ways to reduce the duration of leaf wetting also include: reducing plant stand density, aligning crop rows with the direction of prevailing winds, and irrigating in the late morning or early afternoon (Paibomesai, Celetti, 2012, Hausbeck 2010). Treatment of plants with fungicides can significantly reduce the degree of disease development, however, in studies C.S. Tayviah (2017) reports that *S. vesicarium* is at risk of developing resistance to fungicides because it has a short asexual reproductive cycle, produces several generations per season, and produces many spores by sexual and asexual reproduction, in addition, multiple applications of fungicides are required during each growing season.

To date, onion stemphiliosis is an understudied disease in Ukraine. In this regard, it is relevant to study the species composition of onion pathocenosis, the isolation and determination of the species identity of the causative agents - stemphiliosis, to determine the effectiveness of modern fungicides used on onions.

MATERIALS AND METHODS

Field experiments were conducted in 2019-2021 in the farm AF "Petrodolynske", Odessa district, Odessa region of Ukraine, on the plantings of onions of the variety Banko. The culture was sown in the second decade of March. Soil - southern loamy chernozem, humus content 3.67%, pH 6.7. Seeding rate was 11000 seeds/ha. Seeds germinated 20-22 days after sowing. Weed control was performed with Totril 225 EU herbicide according to the recommendations: the first spraying - in the phase of 1-2 leaves, 1.0-1.5 l/ha; the second spraying as weeds grow, 1.0-1.5 l/ha. In the second half of the growing season we conducted two manual weeding operations.

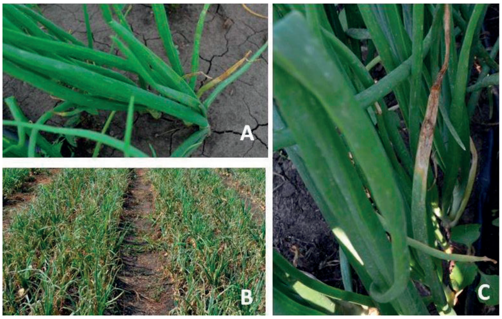

Figure 1. Symptoms of stemphiliosis on onion plants: A - necrotic tips (beginning of the disease); B - general view of affected areas of onion plantings; C - necrotic lesions spreading upwards through the leaves (authors' photos)

To study the species composition of onion mycoflora, we used methods of mycological inoculations on potato-dextrose agar (PDA). Isolates were isolated from leaves of 20-30 bulbs. Small leaf fragments with visible lesions were placed on potato agar and incubated at 22°C for two weeks in a thermostat. After the formation of mycelium, identification of pathogens by morphological features was performed. Identification of phytopathogens was performed according to the methods of V.I. Bilai (1988), N.M. Pidoplychko (1977), N. Shishkoff and J.W. Lorbeer (1989).
Observations of the spread and development of stemphyliosis were carried out dynamically during the growing season, starting from the appearance of the first signs of the diseases. Three leaves on 20 randomly chosen plants from the cutting plot were examined on a scale of 5 points: 0 - no lesions; 1 - up to 10% of surface area affected; 2 - 11 to 25% of surface area affected; 3 - 26 to 50% of surface area affected; 4 - more than 51% of surface area affected.
The disease severity index in all years where the leaves were grouped into classes was calculated as:

$$DSI = \frac{\sum[(class.no)(no.of\ leaves\ in\ each\ class)] \times 100}{(total\ no.leaves\ assessed)(no.classes - 1)}$$

Table 1 shows fungicides and their application rates, which were applied to onions against stemphiliosis. The preparations were applied using a mounted knapsack sprayer with 6 flat jet nozzles, the distance between the nozzles was 50 cm, calibrated for application of 400 l/ha. Experimental plot size is 24 m^2, four-quarter repetition. Leaf lesion counts were started with the appearance of the first signs of the disease, followed before each spraying, and 10-15 days after the last treatment. The experimental data were processed using the method of analysis of variance (Little and Hills, 1991).

Table 1. Fungicides, active ingredients, and consumption rates taken to evaluate efficacy in field trials against Stemphiliosis on onions, 2019-2021

Active ingredient	Trade name	Consumption rates
Diphenoconazole (250 g/l)	Skor, KE	0.5 l/ha
Azoxystrobin (250 g/l)	Quadris, SC	1.0 l/ha
Praclostrobin (67 g/kg) + boscalid (267 g/kg)	Cygnum, VDG	1.5 kg/ha
Ciprodinil (375 g/l) + fludioxonil (250 g/l)	Switch, VDG	1.0 kg/ha
Fluoxastrobin (100 g/l) + prothioconazole (100 g/l)	Fandango 200 EU, KE	1.25 l/ha
Fluopyram (200 g/l) + tebuconazole (200 g/l)	Luna Expirience 400 SC, KS	0.75 l/ha

RESULTS AND DISCUSSIONS

Phytosanitary monitoring has shown that stemphiliosis in the production conditions of southern Ukraine is very common and harmful disease of onion. The development of onion stemphyliosis is observed in onion fields from about mid-June (BBCH 14-15) until harvesting (Figure 1).
At the first stage of work, we studied the species composition of onion pathogenic mycoflora. For this purpose, we selected isolates and determined their species affiliation. For pathogen diagnosis, we used methods of mycological inoculations on potato dextrose agar (PDA). Isolates were isolated in pure culture according to the conventional methods. After germination of fungi, microscopic preparations were made and analyzed under a light microscope at different magnifications. Fungi were identified by morphological features (morphology of spores, spore carriers, etc.) using phytopathological identifiers.
On the green parts isolated from onion plantations, 8 species of fungi were found, among them various species of *Stemphylium* spp, *Stemphylium herbarum* Simm.), which are considered causative agents of onion stemphyliosis and causative agents of other diseases: *Fusarium oxysporum*, onion head mold (*Aspergillus niger, Penicillium*), false powdery mildew or peronosporosis (*Peronospora destructor*), *Alternaria* spp. The data on the morphological features of fungal mycoflora of onions are summarized and presented in Table 2.

Table 2. Cultural characteristics of fungal mycoflora of onions
(STOV "AF Petrodolynskoe, Odessa district, Odessa region, Ukraine, variety Banko)

Exciter	Cultural features of the colony	Peculiarities of morphology
Stemphylium spp.	dirty white, light gray color of colonies, later changing to light brown to dark brown with white and brown stripes.	conidia are olive-brown, oval to ovoid, oblong on conidiophores. The conidia range in length from 14.6 µm to 30.6 µm. The conidia vary in width from 4.7 µm to 15.7 µm.
Fusarium oxysporum	colonies from white to pale lilac, aerial mycelium from almost absent to abundant and fluffy. Abundant macroconidia often form in the central part of the colonies, sometimes producing brownish, blue or purple sclerotia. It often secretes dark purple to dark crimson pigment.	macroconidia spindle-shaped, somewhat curved to almost straight, with 3-5 septa (mainly with 3 septa). Upper cell short, sometimes somewhat hooked, lower cell with stalk or papilla.
Aspergillus niger Thieg.	The fungus forms a loose mycelium. The conidiose zone is black in color	sterigmas, 20×7 microns, 7×3 microns. Conidia globular, 3 microns assembled in chains.
Peronospora destructor	The colonies are white to pale gray, the aerial mycelium is abundant and fluffy.	asexual reproduction occurs with the formation of zoosporangia that grow into a conidia-type growth tube. Dichotomously branching conidiophores are formed. Conidia always sprout hyphae.
Alternaria spp.	gray-purple or pencil-colored velvety plaque of conidial sporulation of the fungus	conidiophores singular 150×3.5 6.5 microns pear-shaped multicellular spores with septa, pencil color, 150×3.5-6.5 microns.
Penicillium Link.	mycelium yellow-green with yellow-white edging, with fine colorless mottling on the surface	conidiophores smooth, 3-4 microns thick, bearing bilobate tassels. Conidia ellipsoidal, slightly narrowed at the end, 3-4 × 2.5-3.5 microns thick, finely bearded

Frequency of occurrence in samples (intensity of spread, %) of isolated pathogens on average was: *Stemphylium* spp. - 13.9%, *Fusarium oxysporum* - 20.7%, *Aspergillus niger* - 18.6%, *Penicillium* - 19,4%, *Peronospora destructor* - 9.5%, *Alternaria spp.* - 17.9% (Figure 2).

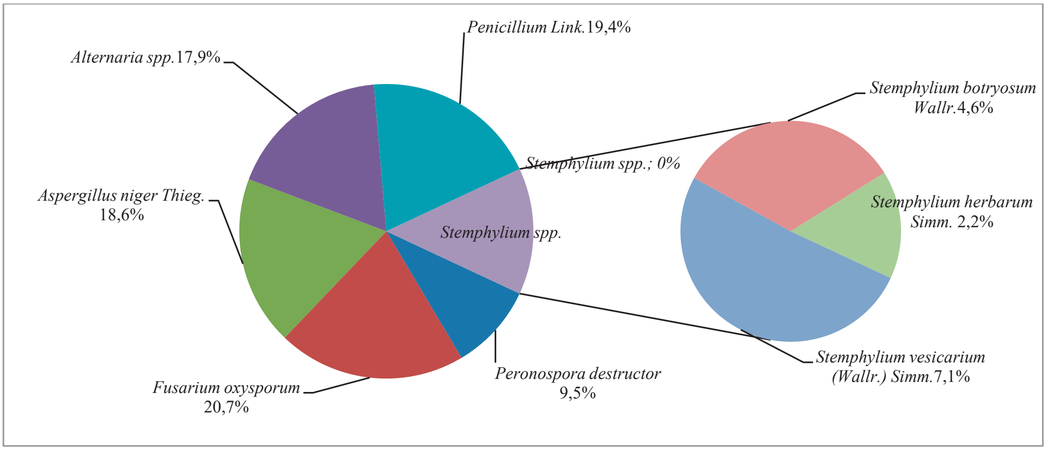

Figure 2. Species composition of fungal mycoflora of onions (% of the number of samples)

Among the eight species of fungi isolated by us, only *Alternaria* and *Stemphylium* are representatives of closely related genera, so they must be identified on the basis of colony culture and conidia morphology (Figure 3).

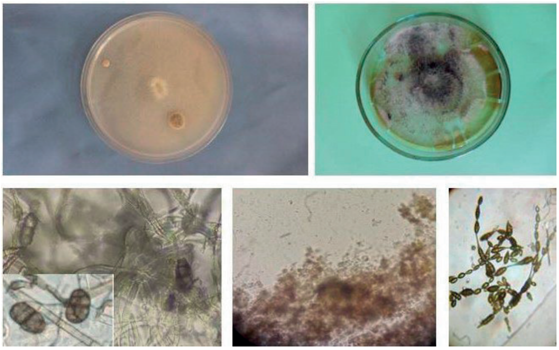

Figure 3. Mycelial colonies on potato dextrose agar (PDA): (A) *Stemphylium* spp.; (B) *Alternaria* spp.; *condyloid* colonies with conidia (C); (D) *Stemphylium* spp.; (E) *Alternaria* spp.

At the second stage, from the spectrum of isolated fungi, we identified the causative agents of onion stemphyliosis. Identification of representatives of the genus *Stemphylium* based on colony culture features and conidia morphology showed the presence of its three representatives in the samples: *Stemphylium vesicarium* (Wallr.) Simm., *Stemphylium botryosum* Wallr., *Stemphylium herbarum* Simm. which are similar in conidia shape, but other characteristics such as size, make them different. Conidia of *S. vesicarium* are oblong or oval with 1-5 transverse septa, sometimes narrowed in the middle, one or three most central of them, and with a complete or nearly complete row of longitudinal septa. The conidia vary in size from 25 to 42 µm in length, 12 to 22 µm in width. *S. botryosum* has spores of subspherical to oblong shape, strongly constricted at the middle septum, 33-35 µm long and N 24-26 µm wide (Shishkoff, and Lorbeer, 1989; Arpita Das et al., 2019). The *S. herbarum* culture was characterized by rapid growth on medium compared to the species described above. The colonies were round in shape, fluffy in texture, and the color of the mycelium ranged from brownish orange to dark brown. The isolated *S. herbarum* produced immature fruiting bodies and many conidia. Spore size range: length 20-30 µm; width 10-15 µm (Lincoln, 2005). The above micromycetes also differed in frequency of occurrence in samples - *Stemphylium vesicarium* (Wallr.) Simm. - 7.1%, *Stemphylium botryosum* Wallr. - 4.6%, *Stemphylium herbarum* Simm. - 2.2%. It is known from the literature that the conditions of the year primarily affect the pathogen, and the degree of this influence depends on its biological characteristics. The maximum lesion of onion with stemphyliosis was noted in 2021 with warm and wet spring and summer periods (Figure 4). However, weather conditions during the growing seasons of 2019 and 2020 were also favorable for disease development (Figure 4). During the 2019 growing season, precipitation was 182.8 mm or 85.4% of the climatic norm for that period (214 mm). The greatest amount of precipitation was observed in the second decade of April (33 mm), the first decade of June (29 mm) and the first decade of August (63 mm). Mean air temperature during the period of tests was 19.6°C, which is 2.4°C above the long-term average (17.2°C). Relative humidity averaged 65%, which was 4% below the multi-year data for this period (69%). Precipitation was 152.0 mm during the growing season in 2020, 29% below the climatic norm for this period. The greatest amount of precipitation was observed in the third decade of May (58.0 mm) and the second decade of June (24.0 mm) in the form of showers. Average air temperature for the period of tests was 18.7°C, which is 1.5°C higher than average multiyear values. Relative humidity averaged 61.0%, which was 8% below the multiyear data for this period. In 2021, precipitation for the growing season was 344.0 mm, 74.0% above the climatic norm for this period. The greatest amount of precipitation was observed in the II decade of May (29.0 mm), I and II decade of June (45.0 and 52 mm) and in the I and III decade of July (39.0 and 60 mm) in the form of showers. Average air temperature during the period of tests was 18.4°C, which is 1.2°C higher than the long-term average. Relative humidity averaged 71.0%, which was 2.0% higher than multiyear data for this period.

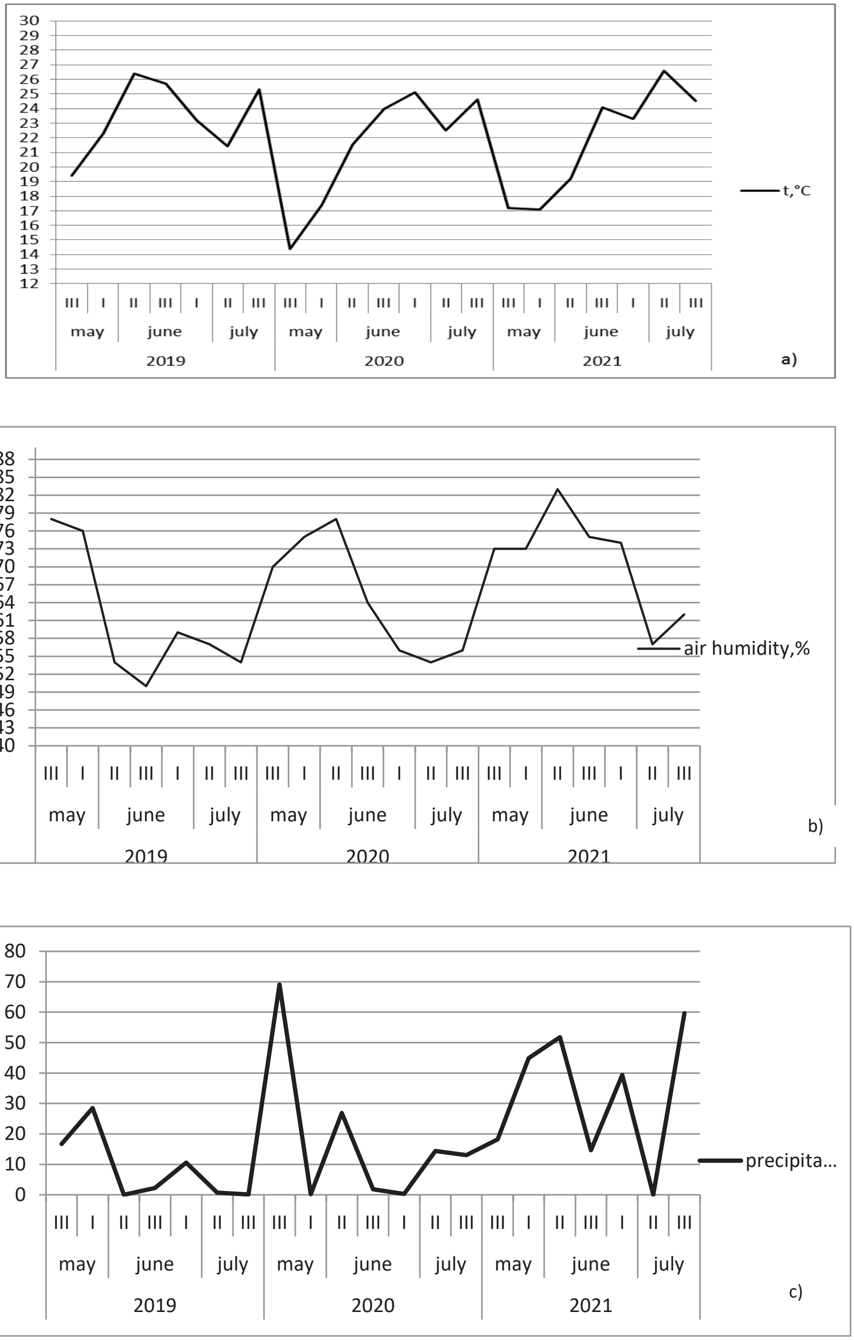

Figure 4. Weather conditions for the growing season of onions, 2019-2021

In onion crops, the disease develops in the following pattern: a low incidence in June (<10%) followed by a rapid increase in July. By late July and early August (during the onion phase of BVSH 43-45), disease incidence can reach 50 to 80% (Figure 5).

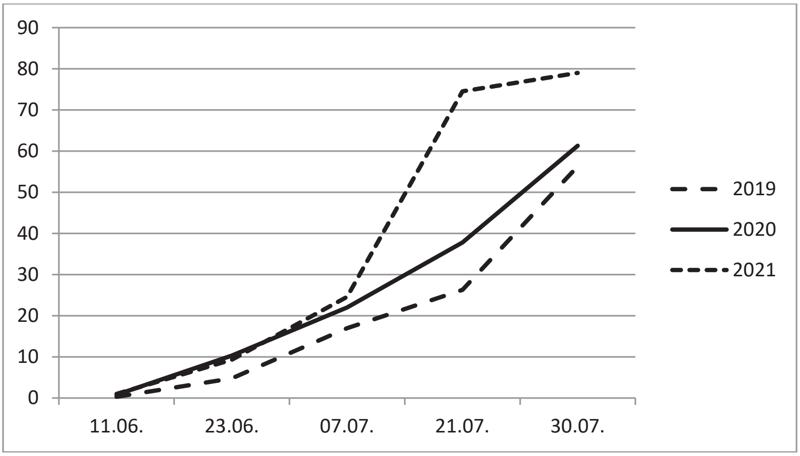

Figure 5. Development of stemphyliosis in onion crops

F. Hay et al. (2021) note that at this time there is a rapid increase in vegetative mass of plants, which contributes to reduction of air movement between plants, increase in its humidity and further development of stemphiliosis. This is usually accompanied by necrosis of the tips and die-off of the outer leaves.

Field studies were conducted in 2019-2021 to determine the most effective fungicides. Five fungicide treatments were conducted throughout the growing season: diphenoconazole (250 g/l), azoxystrobin (250 g/l), pyraclostrobin (67 g/kg) + boscalid (267 g/kg), ciprodinil (375 g/l) + fludioxonil (250 g/l), fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + protioconazole (100 g/l). The first treatment was given at the detection of the first symptoms which were stated in the second decade of June in the onion BBCH 13-14 phase in the form of single spots, the subsequent treatments were given at an interval of 10-14 days, and the last treatment was given at the WWSN 47 stage (the beginning of feather drop: up to 10% of plants were lodged). Diphenoconazole (250 g/l), azoxystrobin (250 g/l), pyraclostrobin (67 g/kg) + boscalid (267 g/kg), ciprodinil (375 g/l) + fludioxonil (250 g/l) were not effective enough. The fungicides fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + prothioconazole (100 g/l) controlled the disease most effectively. Their application during the growing season reduced the lesion of onion plants by 67.1-77.1% and 66.8-79.1%, respectively.

The efficacy of fungicides in the third year (2021) of application decreased compared to 2019 and 2020, which may indicate the emergence of resistance to the tested preparations. Therefore, to develop recommendations for control of onion stemphiliosis, it is necessary to continue studies to evaluate the effectiveness of new fungicides in the field in order to warn producers against using ineffective products.

CONCLUSIONS

Phytosanitary monitoring showed that hemophiliosis in production conditions in the south of Ukraine is a very common and harmful disease of onion. On the control variants the spread of the disease reached at the end of vegetation up to 100.0%, and the intensity of the lesion up to 79.0%.

For the first time in Ukraine, it was found that 8 species of micromycetes belonging to different systematic groups develop on onion. The most frequent are *Peronospora destructor* - 9.5%, *Stemphylium* spp. - 7.1%. The following species dominate by intensity of development: *Fusarium oxysporum* 20.7%, *Aspergillus niger* 18.6%, *Penicillium* 19.4%, *Alternaria* spp. - 17.9%.

From a spectrum of isolated fungi of genus *Stemphylium* spp. we identified species - *Stemphylium vesicarium* (Wallr.) Simm, *Stemphylium botryosum* Wallr, *Stemphylium herbarum* Simm. which had different frequency of occurrence in samples.

It was found that under field conditions, the preparations with the active substances fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + prothioconazole (100 g/l) controlled the disease most effectively.

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IDENTIFICATION OF ALTERNATIVE MEASURES FOR THE MANAGEMENT OF ROOT-KNOT NEMATODES ON SOLANACEOUS VEGETABLE CROPS IN SOUTHWEST BULGARIA

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Abstract

Vegetables from the Solanaceae family (eggplant, tomato, and potato) are among the crops in Europe which in terms of production rank first, and in Bulgaria their production is concentrated in the south-western part. The aim of the study was to identify alternative root-knot nematode control measures applicable in integrated pest management to improve plant health and reduce dependence on chemical pesticides in Solanaceae vegetable production. Based on the data of new research, a summary list of specific combinations of vegetable crops/species of root-knot nematodes in Bulgaria has been compiled. After analysing the problems and according to the innovative practices, methods to control a given root-knot nematode species in a certain crop were indicated. The studies enabled an inventory of potential alternative measures for integrated management and the creation of a dataset that will allow for the improvement of plant health and the reduction of dependence on chemical pesticides in the production of Solanaceae vegetables.

Key words*: alternative measures, root-knot nematodes, Solanaceae vegetable.*

INTRODUCTION

The economic damage caused by plant parasitic nematodes worldwide is estimated at 12.3% (Hassan et al., 2013; Singh et al. 2015). In heavily infected areas, yield losses of more than 50% are observed, in some cases the crop can be destroyed (Nicol et al., 2011). In addition, the presence of nematodes limits the cultivation of several crops on infected areas. Root-knot nematodes (*Meloidogyne* spp.) are distributed throughout the world and belong to the phytonematodes of greatest economic importance, followed by representatives of the genus *Pratylenchus* and the genus *Heterodera* (Ravichandra, 2014). Nematodes of the genus Meloidogyne Goldi 1877 are pests of economic importance for many crops grown in greenhouses and fields (Samaliev and Stoyanov, 2008; Mesa-Valle, et al. 2020). They are obligate, sedentary root parasites, polyphages. The most common species are *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla*, which develop several generations during one growing season and are characterized by high population density and dynamics. The host range of these species includes over 2000 plant species, and species from the Solanaceae family can be defined as the main hosts. Many of the world's economically important agricultural crops belong to the family Solanaceae (*Solanum*, *Capsicum* and *Nicotiana*) with about 28 million hectares under cultivation worldwide (Motti, 2021). Vegetables from the Solanaceae family are among the crops in Europe that rank first in terms of production and production area (Santamaria & Signore, 2021). In recent decades, the consumption of fruits and vegetables has increased, with increased demand for tomato, pepper, and eggplant belonging to the family Solanaceae (Motti, 2021). However, losses are a major problem at all stages from production to consumption. Current chemical nematicides are insufficiently effective and must be optimized for specific pests and crops. The requirements to produce fresh vegetables without residual pollutants increase the interest of growers in the problems related to control measures and managing the impact of biotic stress factors. New and expanding trends in agriculture require research to provide cost-effective and easy-to-use alternatives to conventional synthetic pesticides or to identify measures compatible with integrated programs to protect and minimize the application of chemical agents. Information in the literature regarding the influence of alternative control measures on the development

of vegetable crops to overcome nematode damage is insufficient, there is a lack of specific data on vegetable production in Bulgaria.
The aim of the study was to identify alternative root-knot nematode control measures applicable in integrated pest management to improve plant health and reduce dependence on chemical pesticides in Solanaceae vegetable production.

MATERIALS AND METHODS

The results of the present work were obtained based on the analysis of the scientific literature on the considered problem, the grouping of the obtained data, the monitoring of established root-knot nematode/crop combinations and laboratory analysis of soil and plant samples to determine the species composition of nematodes. The route studies and observations were conducted in the period 2021-2022. In the regions of Sofia, Pernik, Kyustendil, Samokov, Blagoevgrad, Gotse Delchev and Pazardzhik, 22 observation points were selected, which were planted by tomatoes and potatoes during the growing season. According to preliminary information and data of the Bulgarian Agency for Food Safety, root-knot nematodes were found on some of these areas. Investigations of the presence and distribution of root-knot nematodes of genus *Meloidogyne* included greenhouses and field areas of tomatoes, aubergines and potatoes intended for consumption and processing. The leading factor in choosing the plots with potatoes was that they should be grown on agricultural plots where monoculture tobacco was grown in the recent past. Figure 1 shows the surveyed regions.

Figure 1. Regions in Southwest Bulgaria that were subject to root-knot nematode monitoring

RESULTS AND DISCUSSIONS

The following species of root-knot nematodes have been established in Bulgaria: southern root-nematode *Meloidogyne incognita* (Kofoid & White, 1919), peanut root knot nematode *Meloidogyne arenaria* (Neal, 1889), northern root-knot nematode *Meloidogyne hapla* Chitwood, 1949, *Meloidogyne javanica* Treub., 1885, Chitwood, 1949, thames' root-knot nematode *Meloidogyne thamesi* Chitwood, 1952. The cereal root-knot nematode *Meloidogyne naasi* Franklin, 1965 has not been established, but there are conditions for its development.
In Bulgaria in 1925, Chorbadjiev made the first reports of root-knot nematode damage to tobacco seedlings in the region of Shumen District. Later in 1940 Kovachevski found it on cucumbers in the village of Varbitsa, Gorna Oryahovitsa. Trifonova and Gospodinov (1955), Stoyanov (1962), Choleva (1973) and others reported intensive research on the bioecology of the species of the genus *Meloidogyne* (Mateeva, 2004).
The results for the identification of model combinations of vegetable crop/nematode species for the different regions of Bulgaria were presented in a summary list. The data from modern research on the species composition are reflected in Table 1.
The results of our tests confirmed the presence of nematodes of the genus *Meloidogyne* in the studied samples, which confirms their wide distribution of these pests in the region of Southwest Bulgaria. The species *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* were identified. Mixed infection (*M. arenaria* + *M. incognita*) was observed from the greenhouse samples. In the field samples, the dominant species was *M. arenaria*.
The review done here aims to evaluation the latest studies to alternative methods of root-knot nematode control. Table 2 lists those that are easily applicable and effective.

Table 1. Summary list of specific combinations vegetable crop/species of root-knot nematode in Bulgaria

Area	Species of root-knot nematode	Crop	Reference sources
Kresna	*Meloidogyne* spp.,	tomato	Markova L.et al. (2014)
Southern Bulgaria	*Meloidogyne* spp.,	tomato	Trifonova & Vulkova (2007); Voulkova & Trifonova (2009); Tringovska et al. (2015)
	M. arenaria	tomato	Choleva et al. (2005); Yankova et al. (2006); Baycheva et al. (2018)
	Meloidogyne spp.,	greenhouse vegetable crops	Choleva et al. (2004)
Southern Bulgaria	*Meloidogyne* spp.,	greenhouse vegetable crops	Samaliev (2009b)
	M. hapla	potatoes, tomatoes	Trifonova & Voulkova (2008); Markova & Samaliev (2011)
	M. javanica	aubergine	Mohamedova et al. (2016)
	M. incognita	cucumbers, tomatoes	Trifonova & Voulkova (2008); Trifonova & Vachev (2010); Panayotova et al. (2016)
	M. incognita, M. arenaria, M. javanica, M. hapla и *M. thamesi*	greenhouse vegetable crops and arable crops	Samaliev et al. (2018)
	M. arenaria, M. incognita, M. javanica and M. hapla, races 1 and 2 of *M. incognita* and race 1 and 2 of *M. arenaria*	greenhouse vegetable crops	Samaliev (2009a).
Plovdiv, Troyan and Samokov	*M. hapla, M. arenaria* and *M. incognita*	potato	Samaliev & Baicheva (2010); Samaliev & Kalinova (2013)
	M. incognita, M. arenaria and *M. hapla*	over 30 host plants	Stoyanov (1980).
	M. arenaria	cucumbers	Yankova, et al. (2014).

Table 2. Alternative methods for control of root-knot nematodes (*Meloidogyne* spp.) in major vegetable crops

Alternative control measure	Species of root-knot nematode	Crop	Reference sources
cultural practices	*Meloidogyne* spp.	host plants	Azlay et al. (2022)
solarization	*Meloidogyne* spp.	tomato	Yücel et al. (2007); Samaliev (2009b).
resistant varieties	*Meloidogyne* spp., *M. incognita*	tomato	Lizardo et al. (2022); Trifonova & Voulkova (2008); Trifonova & Vulkova (2007); Yankova et al. (2006)
sanitation, heat-based methods	*Meloidogyne* spp.	vegetable crops	Collange et al. (2011)
plant extracts and essential oils	*M. incognita;* *M. incognita* race 2, *M. hapla*	tomato, cucumber,	Abo-Elyousr et al. (2009); Abo-Elyousr et al. (2010); Adegbite (2011); Azlay et al. (2022); Mostafa et al. (2017); Salim et al. (2016); Taniwiryono et al. (2009); Taye et al. (2012); Trifonova & Atanasov (2009); Trifonova (2012)

Alternative control measure	Species of root-knot nematode	Crop	Reference sources
aqueous extracts of garlic and *Ricinus* seeds	*M. incognita*	tomato	El-Nagdi & Youssef (2013)
organic amendment	*M. incognita*	tomato	Asif et al. (2016); Zakaria et al. (2013)
biological, chitinous material, seashell meal	*M. incognita*	tomato	Ladner et al. (2008)
biological management/ nematophagous bacteria and fungi	*Meloidogyne* spp.	host plants	Azlay et al. (2022); Moreira et al. (2015); Singh et al. (2019); Zakaria et al. (2013)
bioproducts of microbial origin	*M. incognita*	tomatoes	Radwan et al. (2012)
commercial products contain *bacteria Bacillus firmus* and *Pasteuria penetrans*, and *Purpureocillium lilacinus* mushroom	*Meloidogyne* spp.	host plants	Lamovšek et al. (2013); Samaliev, H. (1997); Samaliev & Baycheva (2004).
Bacillus thuringiensis /crystal protein/	*Meloidogyne* spp.; *M. arenaria*	tomato	Li et al. (2007); Mohamedova (2009)
rhizobacteria *Bacillus subtilis Bacillus altitudinis* AMCC1040 *Pseudomonas oryzihabitans* and *Xenorhabdus nematophilus*	*M. javanica*	aubergine	Mohamedova et al. (2016)
	M. javanica	host plants	Samaliev et al. (2000)
Fungi antagonists, *Acremonium strictum* и *Trichoderma harzianum*	*Meloidogyne* spp., *M. incognita*	tomato	Goswami et al. (2008); Jindapunnapat et al. (2013); Singh et al. (2019); Trifonova & Vachev (2010)
Nematophagous fungi, *Pochonia chlamydosporia*	*Meloidogyne* spp., *M. arenaria, M. incognita*	organic vegetable production	Atkins et al. (2003); Sosnowska (2007); Trifonova (2014)
stimulating plant growth; rhizobacteria strains	*M. incognita*	tomato	Cetintas et al. (2018)
grafting root stock / *Solanum sisymbriifolium*, cucurbitaceous rootstocks	*M. incognita,* *Meloidogyne* spp.	tomato	Baidya et al. (2017)
cover and biofumigant crops	*Meloidogyne* spp.	tomato and potato	Daneel et al. (2018)
cover crops and green manure crops of the genus Brassica; companion plants	*Meloidogyne incognita,* *Meloidogyne javanica*	vegetable plasticulture	Monfort et al. (2007); Stirling & Stirling (2003); Tringovska et al. (2015)
antagonistic plants (sorghum, crotalaria, mucuna, guandu bean and neem	*M. javanica*, *M. enterolobii*, *Meloidogyne* spp., *M. arenaria*	host plants,	Moreira et al. (2015); Yasmin et al. (2003); Al Body & Mateeva (2007).
African marigold, *Tagetes erecta*	*M. incognita*, *M. arenaria*	tomato, greenhouse vegetable	Natarajan et al. (2006); Al Body & Mateeva (2007)

Alternative control measure	Species of root-knot nematode	Crop	Reference sources
Tagetes minuta, *Datura metel*, *D. stramonium* and *Ricinus communi*	*Meloidogyne* spp.	tomato	Oduor-Owino (2003)
abamectin	*Meloidogyne* spp.	tomato	Qiao et al. (2012);
Chlorella vulgaris /algae/ and potassium húmate	*M. arenaria*, *M. incognita*, *M. hapla*	greenhouse tomato	Choleva et al. (2007); Choleva et al. (2004)
fertilization, compost, fertilizers, biostimulators	*Meloidogyne* spp., *M. javanica, M. incognita*	greenhouse tomato	Saeedizadeh et al. (2020); Markova, L. et al. (2014)
poultry manure	*Meloidogyne* spp.	tomato	Chindo & Khan (1990)
silver nanoparticles	*Meloidogyne* spp.		Bernard Monfort et al. (2019);
selenium	*M. arenaria*	tomato	Baycheva et al. (2018)

After analysis of literature data, we found large variations between studies. Many practices were listed as only partially effective; therefore, combining control methods in integrated nematode management is a challenge that requires a systematic approach and identification of key future research. There is growing concern among vegetable growers as registered chemical nematicides decline. Alternative methods, means and techniques based on innovative practices are needed to solve the problem.

Despite the availability of effective alternative methods for root-knot nematode control, limited progress has been made in their implementation. In part, this may be due to the fact that the plant parasitic nematodes control has traditionally been carried out primarily by chemical means.

The trend worldwide for a chemical pesticide-free agricultural production and environment minimizes the use of pesticides (Lykogianni et al., 2021). The application of crop rotation is economically unprofitable for farmers, and the main part of greenhouse vegetables in Bulgaria is grown as a monoculture. The breeding of resistant varieties against these enemies is a difficult task (Samaliev & Stoyanov, 2008). This necessitates the search for alternative methods of control of *Meloidogyne* spp. In this aspect, among the non-chemical control measures is the biological method, especially in the context of integrated plant management. One of the many options for biological control is to specifically obtain and support or colonize biological active agents that suppress the development of plant parasitic nematodes. In this regard, the availability of different types of biological agents with potential for successful control of these enemies is of interest. Nematode antagonists can be various types of bacteria, fungi, viruses, rickettsia, predatory nematodes such as mites, collembolans (Volpiano et al., 2019). Trifonova et al. (2009) conducted mycological studies in the southern regions of Bulgaria and found that *Fusarium oxysporum*, *Verticillium chlamydosporium* and *Gliocladium roseum* parasitized the eggs of female individuals of the genus *Meloidogyne*, with 7.6% to 23.5% of the eggs in subsequent generations died. Of the previously known microorganisms with an antagonistic effect against Meloidogyne, spp. non-pathogenic bacterial strains have increasingly been used in recent years (Sidhu, 2018). The use of rhizobacteria to biologically control plant diseases and to stimulate plant growth has been practiced with great success (Shaikh & Sayyed, 2015; Verma, 2019). The search for suitable rhizobacteria against plant-pathogenic nematodes started about 30 years ago (Zavaleta-Meija & VanGundy, 1982; Mohamedova & Samaliev, 2011; Sidhu, 2018), mainly the genera *Pseudomonas* spp. and *Bacillus* spp. They have properties that determine their success in practice - they inhibit egg hatching or produce metabolites toxic to nematodes but have no negative effects on soil and on plant growth and development. Studies carried out by Ahmed (1999) on the effect of the rhizobacterium *Bacillus subtilis* on damage caused by *Meloidogyne* spp. in tomato showed that even when the root-knot nematode population increased and damage was important, plant growth was enhanced (induced tolerance). There is data in the literature that suggests that treatment of plants with synthetic phytohormones and organomineral fertilizers also induced resistance to pests including nematodes (Guimarães, 2010; Bhattacharya, 2021; Dar et al., 2021).

In greenhouse experiments, the influence of using the following fertilizers Kendal, 18 Biopower, Nutriphite, Hortiplus MIQL 2826, Max Fitus as inducers of resistance to *Meloidogyne incognita* was studied. The authors reported proven differences in nematode population numbers between the control and variants treated with Biopower and Nutriphite fertilizers (Assunção et al., 2010).

In Bulgaria, Markova et al. (2014) studied the effect of the liquid root biostimulator Fertiactyl® GZ on damage by root-knot nematodes *Meloidogyne* spp. in tomato plants grown in greenhouse conditions.

The authors found that the liquid root biostimulator had a stimulatory effect on the growth and development of plants infected with Meloidogyne spp., and the tested tomato cultivars Raleigh and Matthias after treatment with Fertiactyl® GZ showed no symptoms of damage.

The cultivation of resistant varieties as well as the observance of crop rotation are methods that limit the use of the chemical control method. The protection of vegetable crops of the Solanaceae family against plant parasitic nematodes should ensure the health and potential of the crops through the extensive implementation of alternative control measures such as sanitary measures, phytosanitary monitoring, crop rotation, mixed cropping, breeding of resistant or tolerant varieties, application of biological agents and other innovative practices. The emergence of nematode resistance to nematicides, the negative impacts on human health and the environment, and the drastic decrease in the availability of existing and new chemical pesticides in Europe lead to an increased need for alternative control options. In addition, there are legal requirements to regulate agricultural production according to IPM principles and to market plant products with little or no pesticide residues (Directive 2009/128/EC).

CONCLUSIONS

The analysis of the species composition of root-knot nematodes in solanaceous vegetable crops in Southwest Bulgaria showed that the distribution and the population structure were relatively constant over time.

Limited progress has been observed in implementing alternative measures to control root-knot nematodes.

A range of data was created to enable the inclusion of alternative measures in integrated pest management (IPM) programs and to reduce reliance on chemical control.

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THE ROLE OF THE PARENTAL FACTOR IN THE MANIFESTATION OF GROWTH AND DEVELOPMENT TRAITS AT THE F_1 TOMATO HYBRIDS

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Abstract

The paper presents the results of assessing the resistance of some parent varieties and reciprocal F_1 combinations of tomatoes to stressful (40°, 42℃) and optimal (25℃) temperatures. The analysis of the variability of the resistance character was carried out based on the length of the embryonic radicle, stem and whole seedling. In most of the cases stressful temperatures produced significant inhibition of growth organs. The differences in the manifestation of the analyzed characters in the reciprocal F_1 hybrids both in the control variant and in the variants with stressful temperatures demonstrate the involvement of the parental factor in their phenotype on the background of different temperatures. The maternal effect was more pronounced at the temperature of 42℃ than under optimal conditions. Manifesting of the parental entity effect on the degree and orientation of dominance reveals its influence on the allelic interactions of the F_1 heterozygous genotype. The overdominance in relation to the best parent indicates that the parental entity intensifies the influence of the recessive alleles on those dominant, involved in the control of growth characters of tomato plants.

Key words: *tomato, temperature, variability, parental effect, dominance.*

INTRODUCTION

Climate change most directly influences the productivity and quality of fruits, and thermal stress is a major abiotic factor that worldwide limits the productivity of crops, including tomatoes, thus presenting a problem for food security (Battisti, 2009; Asseng et al., 2011; Bita & Gerats, 2013; Tripathi et al., 2016; Bisbis et al., 2018). Although the extent of climate changes cannot be exactly predicted, the prognosis of the specialists in the field take into account that we can expect a higher frequency of extreme weather events, with the associated risks and damages becoming more significant (Van & Darriet, 2016).
Based on several scenarios, by the end of the 21st century global temperatures are expected to increase by an average of 1-3.7ºC above their 1986-2005 levels (IPPC, 2014). The challenges generated by climate change will thus require the implementation of appropriate and cost-effective strategies to adapt newly created varieties in a timely manner to local conditions for an effective risk reduction (Fraga et al., 2012; Porter et al., 2014; Bisbis et al., 2018).
Although tomatoes are grown in different ecological and geographical areas, they are particularly sensitive to high temperatures. The optimum temperature for tomato cultivation is 25-30°C during the day and 20ºC at night (Camej et al., 2005; Ribeiro et al., 2008; Carvalho et al., 2011).
The increase of a few degrees from the optimal temperature can greatly affect the reproductive organs, especially the viability of pollen, the development of gametes and the pollination capacity, as a consequence the productivity decreases considerably (Peet et al., 1997; Sato et al., 2000; Firon at al., 2006). High temperatures can cause significant productivity losses and damage to fruit quality (Nahar & Ullah, 2011). The wide spread of highly productive tomato varieties created in the Republic of Moldova is affected by the increasingly fluctuating biotic and abiotic conditions, specific to this area in recent decades.
The incorporation of genetic resistance in crop plants is considered the most effective and sustainable method of reducing the effects of limiting conditions. For the intended purpose, it is necessary to know the genetic basis of the reaction to adverse factors. It should be noted that due to its quantitative nature, resistance depends on a series of factors, among which we

can mention genotypic, environmental factors and *genotype x environment* interactions.

The complex genetic determinism of quantitative characters, including resistance to heat, makes it difficult to succeed in the breeding activity, whose goal is to create genotypes that combine several valuable characters. It is known that quantitative traits are more easily improved in the case of their high heritability, and their genetic variability is important for expanding the genetic background that makes breeding programs more efficient (Taneva et al., 2019) and for identifying parents that will generate transgressive segregations (Patro & Ravisankar, 2004). The maternal factor is often involved in epigenetic phenomena, associated with the modification of gene expression (Richards, 2006; Bird, 2007) and greatly affect the phenotype (Bossdorf, 2008).

The maternal form is a physiological environment for the development of the embryo and the seed and can influence the germination, competitiveness and/or fertility of the offspring (Wolf, 2000; Latzel, 2009), the quality and size of the seeds, thus determining the growth and development potential of the offspring plants (Sills, 1995).

The aim of our research was to determine the effect of the parental factor on the tomato genotype x temperature interactions, the degree of dominance and the cluster organization of the parents and F_1 hybrids, based on growth and development characters.

MATERIALS AND METHODS

As initial material for the intended researches, 5 reciprocal F_1 hybrid combinations were used: Dolgonosic x Mary Gratefully/Mary Gratefully x Dolgonosic, Flacara x Vrojainii/ Vrojainii x Flacara, Flacara x Desteptarea/ Desteptarea x Flame, L 10B x Rufina/Rufina x L 10B, Rufina x Flacara/Flacara x Rufina and 7 parental forms: Rufina, Dolgonosic, Flacara, L 10B, Vrojainii, Mary Gratefully, Desteptarea.

The testing of the tomatoes reaction to high temperature was carried out under controlled conditions. Seeds of parental forms and F_1 hybrids were placed in Petri dishes between 2 sheets of filter paper moistened with 6 ml of distilled water. For each genitor/F_1 hybrid, 9 Petri dishes with 30 seeds were used, of which 3 boxes were kept constant for 7 days at the optimal temperature of 25°C (control). In the case of stressful temperatures variants, the seeds were initially maintained for 3 days at the optimal temperature, on the 4th day they were transferred to thermostats with temperatures of 40° and 42°C, 3 dishes in each for 6 hours, after which they were returned to optimal temperature conditions until the 7th day. The growth capacity of the samples at different temperatures (25°, 40°, 42°C) was established based on the length of the embryonic radicle, stem and integral seedling (Mihnea, 2017).

The degree of dominance (h_p) was established based on the formula proposed by Brubaker (1966):

$h_p = F_1 - 0{,}5\ (P_1+P_2)/\ H_p - 0{,}5\ (P_1+P_2)$,

where: F_1 - the average value of the character in the F_1 generation;

P_1, P_2 - the average value of the character in the parental forms;

H_p - the average value of the character evaluated at the best parental form.

The effect of reciprocity was calculated according to the formula:

$r_e = (b - a)/(B - A)$,

where: A and B - character values for the parental forms involved in crossing; a - for the ♂A x ♀B hybrid; b - for the reciprocal hybrid ♂B x ♀A. The positive value r ($r > 0$) signifies the paternal effect, and negative ($r < 0$) - maternal, the absolute value r ($|r|$) shows the relative appreciation of these effects in units, equal to the differences in the character values of the parental forms (B - A) (Reinhold, 2002).

The cluster analysis of the degree of similarity/difference of tomato genotypes based on growth and development characters at different temperatures was performed based on the iterative algorithm for building dendrograms and the *k*-means centroid method - methods successfully used in genetics and breeding research (Lupaşcu et al., 2019; Kanavi, 2020).

The obtained data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

The analysis of the reaction of some parent varieties and reciprocal F_1 hybrids in response to the influence of different temperature levels on the growth characters of tomatoes in early ontogenesis, demonstrated that the reaction of the plants to the 3 temperature levels (25°C - optimal, 40° and 42°C - stressful) was differentiated - specific to the genotype, the hybrid, the crossing orientation, the analyzed character: the length of the radicle, stem and seedling.

The length of the radicle. In the parental forms, it was found that in optimal conditions the character varied within the limits of 24.2-49.2 mm, at 40°C - 12.4-36.9 mm, 42°C - 16.2-39.3 mm (Figure 1A). The degree of inhibition of the growth of the parental forms under the influence of the temperature 40°C constituted 14.1-56.4%, and of the temperature 42°C - 22.8-61.4%, compared to the optimal conditions. Significant inhibition of radicle growth was observed in genotype L 10B (61.4%) and stimulation of 7% in variety Rufina. In the case of reciprocal F_1 hybrids, a strongly differentiated reaction was manifested in Dolgonosic x Mary Gratefully: -49.0 ... +7.0% and Flacara x Vrojainii: -9.1 ... -30.8%, and the most insignificant – in Vrojainii x Flacara: - 0.8...-1.1%.

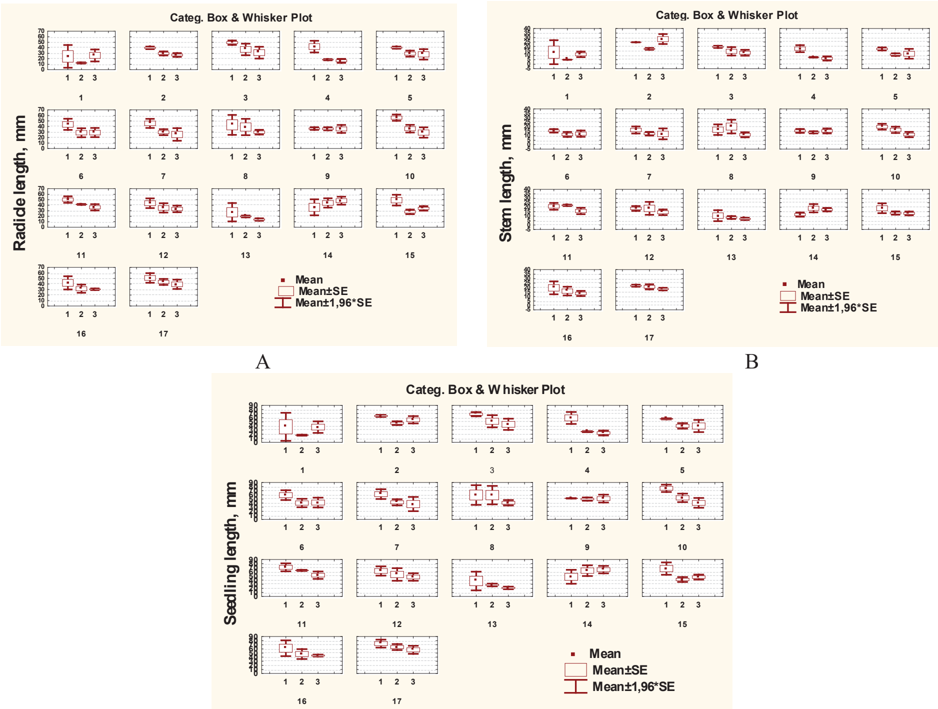
Stem length. In the control variant, the character varied within the limits of 13.5 ... 24.6 mm (Figure 1B). The temperature of 40°C caused growth inhibition which was 18.4-62.2% of the control. Significant repressions were recorded in the variety Rufina (62.2%) and the line L 10B (55.2%). The degree of inhibition in the hybrid combinations varied within wide limits - 2.1 ... 26.5%. Lack of reaction showed the combination F_1 L 10B x Rufina, and significant stimulations - F_1 Flacara x Vrojainii, F_1 Rufina x Flacara (Figure 1 B).

The reaction of the tomato stem at the temperature of 42°C was different: lack of sensitivity to the F_1Vrojainii x Flacara combination and stimulation to the Dolgonosic variety, the F_1 Rufina x Flacara hybrid - 14.6 and 47.0%, respectively. The strongest influence of temperature on the length of the stem was attested to L 10B, F_1 Flacara x Desteptarea: - 62.6 and -43.5%, respectively (Figure 1 B).

Seedling length. It was observed that the temperature of 40°C produced the decrease of the character in the parental forms by 11.5...56.3%, and in the reciprocal hybrids by 0.5...40.7% of the control. Pronounced decrease in seedling length was recorded in the variety Rufina, line L 10B, hybrids F_1 Flacara x Desteptarea, F_1 Rufina x Flacara which varied within the limits: 39.1... 56.3%. Less sensitive was Desteptarea: -11.5%, F1Vrojainii x Flacara: -3.7%, F_1 Desteptarea x Flacara: - 10.7%, compared to the control (Figure 1C). It was found that the temperature of 42°C in the most of the cases inhibited the growth of the seedling. For example, in L 10B and F_1 Rufina x L 10B the inhibition was 61.7 and 44.7% of the control. Only in the F_1 Rufina x Flacara combination was stimulation recorded, which constituted 36.4%.

It should be noted that in some cases, there were significant differences in the indices analyzed in the reciprocal hybrids, both in the control variant and in the variants with stressful temperatures. For example, both in the case of the length of the radicle, stem and seedling, in the control version, in the hybrids F_1 Rufina x Flacara, F_1 Rufina x L 10B, the length of the radicle was 26.4 and 37.5%, the length of the stem - 37.8 and 43.5%, and of the seedling - 29.4% and 39.2, respectively, lower than in the reciprocal analogues. Significant differences were also found in variants with stressful temperatures. Thus, in the case of temperatures of 40°C and 42°C, significant differences between the reciprocal analogues were found in the combinations F_1 Flacara x Rufina, F_1 Rufina x L 10B (Figure 1 A, B).

The differences manifested in reciprocal F_1 hybrids demonstrate the involvement of the parental factor as an entity of hybridization components in the formation of growth characters under the influence of different temperatures.



A B

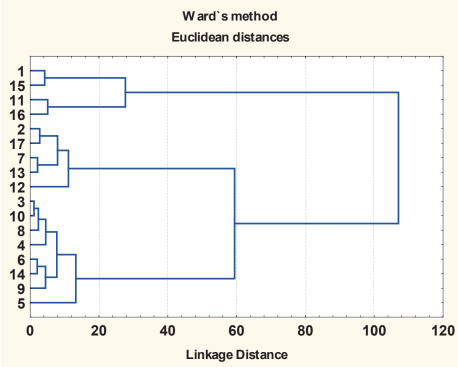
C

Figure 1. The influence of temperature on the length of the radicle (A), the stem (B) and the intact tomato seedling (C)
Horizontally: 1 - Control (25°C); 2 - 40°C; 3 - 42°C
1 - Rufina, 2 - Dolgonosic, 3 - Flacara, 4 - L 10B, 5 -Vrojainii, 6 - F_1 Dolgonosic x Mary Gratefully, 7 - F_1 Mary Gratefully x Dolgonosic, 8 - F_1 Flacara x Vrojainii, 9 - F_1 Vrojainii x Flacara, 10 - F_1 Flacara x Desteptarea, 11 - F_1 Desteptarea x Flacara, 12 - F_1 L 10B x Rufina, 13 - F_1 Rufina x L 10B, 14 - F_1 Rufina x Flacara, 15 - F_1 Flacara x Rufina, 16 - Mary Gratefully, 17 – Desteptarea

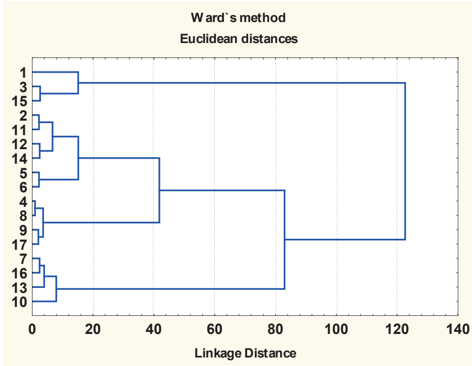
Cluster analysis by constructing dendrograms based on the agglomerative-iterative algorithm demonstrated that the tomato genotypes differed significantly based on the reaction of the embryonic radicle, stem and seedling both under optimal and stressful conditions. The distribution based on Euclidean distances highlighted the formation within the evaluated set of genotypes of distinct clusters: 3 under optimal conditions (Figure 2 A) and 4 under stressful conditions (Figure 2 B, C).

The degree of similarity between tomato genotypes according to the reaction of growth organs at different temperature levels was different. For example, under optimal conditions, genotypes 3, 10; 2, 17; 7, 13, recorded the highest similarity, confirmed by the smallest Euclidean distance for all evaluated characters. The differences between these genotypes were accentuated in the case of stressful temperatures, which led to their location in different clusters.

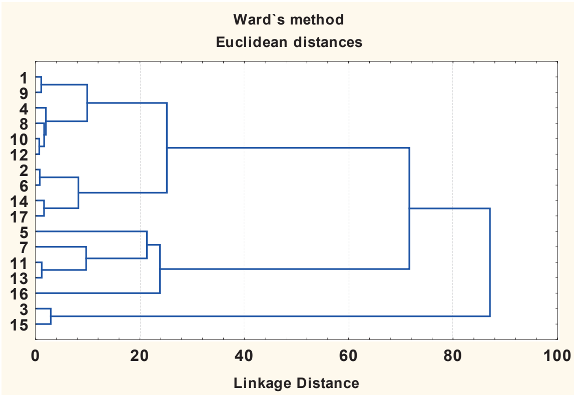
It was found that the intercluster variance was higher than the intracluster variance at all 3 temperatures, which indicates that the tomato F_1 parents and hybrids were successfully clustered based on the 3 characters under study. Through the ratio of intercluster variance to intracluster variance, it was found: 1) the length of the stem showed a diminished discriminatory capacity compared to other 2 characters; 2) the variability of the tested genotypes decreased at the temperature of 42°C, which could be explained by the fact that this temperature level approaches the limits of the adaptive possibilities of tomato plants. Thus, the most successful differentiation of varieties and F_1 hybrids occurred at the temperature of 40°C (Table 1).



A



B



C

Figure 2. Distribution dendrogram of F_1 tomato varieties and hybrids based on growth characteristics in optimal conditions: A - 25°C and thermal stress, B - 40°C, C - 42°C

1 - Rufina, 2 - Flacara, 3 - L 10B, 4 - Vrojainii, 5 - Dolgonosic, 6 - Mary Gratefully, 7 - Desteptarea, 8 - F1 Dolgonosic x Mary Gratefully, 9 - F_1Mary Gratefully x Dolgonosic, 10 - F_1Flacara x Vrojainii , 11 - F_1 Vrojainii x Flacara, 12 - F_1 Flacara x Desteptarea, 13 - F_1 Desteptarea x Flacara, 14 - F_1 L 10B x Rufina, 15 - F_1 Rufina x L 10B, 16 - F_1 Rufina x Flacara, 17 - F_1 Flacara x Rufina

Table 1. Analysis of inter- and intracluster variance in the interaction of tomato genotypes with different temperature levels

Variant	Intercluster variance	df	Intracluster variance	Ratio	df	F	p
25°C							
The length of the radicle	894.788	2	171.4969	5.22	14	36.52262	0.000003
The length of the stem	122.963	2	84.587	1.45	14	10.17574	0.001868
Seedling length	1703.801	2	220.3010	7.73	14	54.13780	0.000000
40°C							
The length of the radicle	1101.836	2	158.1685	6.97	14	48.76352	0.000000
The length of the stem	305.844	2	72.7750	4.20	14	29.41816	0.000010
Seedling length	2576.107	2	333.6302	7.72	14	54.05012	0.000000
42°C							
The length of the radicle	705.529	2	309.4120	2.28	14	15.96158	0.000245
The length of the stem	256.028	2	122.7210	2.09	14	14.60385	0.000375
Seedling length	1764.502	2	256.9770	6.87	14	48.06466	0.000001

By classifying the genotypes based on the 3 characters, it was found that in the control variant cluster 2 met 3 genotypes - 1, 15, 16 with the lowest values of the analyzed characters, and cluster 3 - the genotypes with the highest values (Table 2).

Table 2. Descriptive cluster analysis

Cluster	Character	x	Genotype
	Control		
1	The length of the radicle, mm	41.79	3 – L 10B, 4 – Vrojainii, 5 – Dolgonosic, 6 – Mary Gratefully, 8 – F_1 Dolgonosic x Mary Gratefully, 9 – F_1 Mary Gratefully x Dolgonosic 10 – F_1 Flacara x Vrojainii, 11 – F_1Vrojainii x Flacara, 14 – F_1 L 10B x Rufina
	The length of the stem, mm	17.92	
	Seedling length, mm	59.69	
2	The length of the radicle, mm	29.30	1 – Rufina, 15 – F_1 Rufina x L 10B, 16 – F_1 Rufina x Flacara
	The length of the stem, mm	11.90	
	Seedling length, mm	41.20	
3	The length of the radicle, mm	51.08	2 – Flacara, 7 – Desteptarea, 12 – F_1 Flacara x Desteptarea, 13 – F_1 Desteptarea x Flacara, 17 – F_1 Flacara x Rufina
	The length of the stem, mm	19.84	
	Seedling length, mm	71.34	
	Temperature 40°C		
1	The length of the radicle, mm	16.80	1 – Rufina, 3 – L 10B, 15 – F_1 Rufina x L 10B
	The length of the stem, mm	7.10	
	Seedling length, mm	23.87	
2	The length of the radicle, mm	32.03	2 – Flacara, 4 – Vrojainii, 5 – Dolgonosic, 6 – Mary Gratefully, 8 – F_1 Dolgonosic x Mary Gratefully, 9 – F_1Mary Gratefully x Dolgonosic, 11 – F_1Vrojainii x Flacara, 12 – F_1 Flacara x Desteptarea, 14 – F_1 L 10B x Rufina, 17 – F_1 Flacara x Rufina
	The length of the stem, mm	14.45	
	Seedling length, mm	46.48	
3	The length of the radicle, mm	42.13	7 – Desteptarea, 10 – F_1Flacara x Vrojainii, 13 – F_1 Desteptarea x Flacara, 16 – F_1 Rufina x Flacara
	The length of the stem, mm	20.45	
	Seedling length, mm	62.63	
	Temperature 42°C		
1	The length of the radicle, mm	29.64	1 – Rufina, 2 – Flacara, 4 – Vrojainii, 6 – Mary Gratefully, 8 – F_1 Dolgonosic x Mary Gratefully, 9 – F_1 Mary Gratefully x Dolgonosic, 10 – F_1 Flacara x Vrojainii, 12 – F_1 Flacara x Desteptarea, 14 – F_1 L 10B x Rufina, 17 – F_1 Flacara x Rufina
	The length of the stem, mm	12.29	
	Seedling length, mm	41.94	
2	The length of the radicle, mm	15.10	3 – L 10B, 15 – F_1 Rufina x L 10B
	The length of the stem, mm	6.70	
	Seedling length, mm	21.85	
3	The length of the radicle, mm	37.22	5 – Dolgonosic,7 – Desteptarea, 11 – F_1Vrojainii x Flacara, 13 – F_1 Desteptarea x Flacara, 16 – F_1 Rufina x Flacara
	The length of the stem, mm	18.96	
	Seedling length, mm	56.18	

In the variant with temperature 40°C, 3 genotypes - 7, 10, 13, 16, and in the case of temperature regime 42°C 5 of the genotypes – 5, 7, 11, 13, 16 formed cluster 3, with the highest values of the evaluated characters. From the data obtained we can conclude that genotypes 7, 13 and 16 show complex resistance to the mentioned temperature stresses and are of interest in their use as sources of resistance to unfavorable temperatures. The processing of the experimental data through factorial analysis of variance allowed the appreciation of the variability and the degree of influence of temperature, genotype and their interaction in the weight of the phenotypic manifestation of the growth and development characters of the investigated tomato genotypes (Table 3).

Table 3. Bifactorial analysis of tomato *genotype x temperature* relationships

Source of variation	Freedom degree	The length of the radicle		The length of the stem		Seedling length	
		Mean sum of squares	Contribution in the source of variation, %	Mean sum of squares	Contribution in the source of variation, %	Mean sum of squares	Contribution in the source of variation, %
Genotype	16	448.3*	15.8	125.7*	32.8	913.3	18.1
Temperature	2	2231.9*	79.0	216.8*	56.5	3823.9	75.9
Genotype x temperature	32	89.7*	3.2	27.9*	7.3	186.1	3.7
Random effects	102	56.8	2.0	13.0	3.4	117.1	2.3

*- $p<0.05$.

The mean sum of squares for the 3 characters analyzed was the highest in the case of temperature as a source of variation: 2231.9 - radicle, 448.3 - stem, 3823.9 - whole seedling, followed by genotype and *genotype x temperature* interactions.

By calculating the percentage weight in the source of character variation, it was found that the contribution of genotype, temperature and *genotype x temperature* interactions for radicle length was 15.8, 79.0, 3.2%; stem - 32.8, 56.5, 7.3% and seedling - 18.1, 75.9, 3.7%. So, the variability of the lengths of the embryonic radicle, stem and seedling depends the most on temperature, although the role of the genotypic factor is not negligible, which constituted 15.8, 32.8, 18.1%, respectively, of the mentioned characters. Thus, stem length depended on genotype to a greater extent than other two characters under study. The length of the whole seedling was largely determined by the length of the radicle, this being confirmed by the comparable weight (15.8, 18.1%) of the genotype in the source of variation of these characters. From the data presented, it can be seen that the role of *genotype x temperature* interactions was not relevant (2.0-3.4%) (Table 3).

The research of the role of the parental factor on the growth of tomato radicle, stem and seedling under optimal conditions (25°C), demonstrated that in all variants, except for the combination Dolgonosic x Mary Gratefully / Mary Gratefully x Dolgonosic for stem length and seedling length, Flame x Awakening/ Awakening x Flame for radicle and seedling length, the stronger influence of paternal form was manifested. It should be noted that compared to the control variant, the role of the maternal factor was more pronounced at 42^0C, the effect of reciprocity registering values of -1.97...- 4.92 for the radicle length; -1.6... -3.64 – stem length; -1.87...-2.65 - seedling length (Table 4).

Table 4. Parental effect on tomato growth organs under optimal and stressful conditions

F_1 hybrid	The length of the radicle			The length of the stem			Seedling length		
	25^0C	40^0C	42^0C	25^0C	40^0C	42^0C	25^0C	40^0C	42^0C
Dolgonosic x Mary Gratefully	+0.82	+0.56	-3.01	-0.18	-0.67	+0.02	-0.89	+1.8	+0.32
Flacara x Vrojainii	+0.77	+0.42	-4.92	+0.54	+1.73	-3.64	+0.7	+0.86	-2.43
Flacara x Desteptarea	-3.4	+0.81	+0.87	+0.36	+0.89	+0.81	-1.12	+0.84	+0.85
L 10B x Rufina	+0.92	+2.61	-1.97	+2.08	+3.70	-1.61	+1.12	+3.01	-1.87
Rufina x Flacara	+0.52	-0.67	-2.74	+1.18	-0.57	-2.33	+1.47	-0.64	-2.65

The data obtained show the need to take into account the orientation of hybridization component crosses in tomato in order to make the most successful use of the biological potential of the parents for the creation of resistant forms. The parental factor also influenced the degree of dominance (h_p) of growth characters. For example, in the control variant h_p for the length of the radicle varied within the limits of -1.9...+7.25, and in the variants with temperatures of 40, 42°C - within the limits of -2.1...+6.79 and -1.67...+7.84 - i.e. between the predominance of the parent with the highest radicle growth values - to the predominance of the parent with the lowest values, which denotes the existence of specific interactions of the alleles of the two genomes, function of the character, combination and temperature level.

In the control variant, the degree of dominance of the stem length character varied within wide limits - from positive overdominance (+1.63) to negative overdominance (-2.64). In variants with stressful temperatures, the rate of combinations with positive overdominance increased. Thus, if in optimal conditions the positive predominance was registered with a combination - L 10B x Rufina (+1.63), at the temperature of 40^0C it was registered with 5 hybrids (+1.45 ... +9.0), and at the temperature of 42^0C - with 4 hybrids (+1.11 ... +5.78). Regarding seedling length in the control variant, the degree of dominance varied within the limits: -6.14...+ 1.25, and in the variant with stressful temperatures: -23.7...+7.60 and -1.53... +6.52 to the 40^0C and 42^0C temperatures, respectively (Table 5).

It should be noted that at the reciprocal hybrids, differences in the degree of dominance were found in terms of the level of manifestation, as well as the orientation of dominance, which largely depends on the orientation of the cross, the tested organ and the temperature level. In the variant with stressful temperatures, in most cases the differences between reciprocal hybrids were stronger than under optimal conditions.

For example, out of 5 pairs of reciprocal hybrids only in the combinations Dolgonosic x Mary Gratefully/Mary Gratefully x Dolgonosic the differences for stem length were insignificant both under optimal and stressful conditions, in the other F_1 reciprocal hybrids the values, but sometimes also the h_p orientation have were different, which demonstrates once again the influence of the parental factor on the phenotype of tomato root, stem and seedling (Table 5).

Table 5. The influence of the parental factor on the degree of dominance of growth and development indices in tomato at different temperatures

F_1 hybrid	The length of the radicle			The length of the stem			Seedling length		
	25°C	40°C	42°C	25°C	40°C	42°C	25°C	40°C	42°C
Dolgonosic x Mary Gratefully	+2.63	-2.1	+0.22	-2.64	-8.67	-1.15	+0.20	-23.7	-1.48
Mary Gratefully x Dolgonosic	+4.27	-1.0	-1.67	-2.28	-7.3	-1.19	+0.59	-17.7	-2.11
Flacara x Vrojainii	-0.40	+1.55	+0.07	-1.3	+4.17	-2.17	+0.15	+2.41	-0.57
Vrojainii x Flacara	-1.90	+0.79	+4.0	-2.4	+0.61	+5.17	-0.61	+0.68	+2.86
Flacara x Desteptarea	+7.25	-1.26	-1.49	-0.67	-0.32	-1.59	-6.14	-0.9	-1.53
Desteptarea x Flacara	+0.13	+0.35	+0.24	+0.08	+1.45	+0.04	+0.19	+0.86	+0.16
L 10B x Rufina	+1.19	+6.79	+2.49	+1.63	+9.0	+2.39	+1.25	+7.60	+2.48
Rufina x L 10B	-0.65	+1.48	-1.43	-2.63	+7.0	-0.8	-1.01	+1.5	-1.28
Rufina x Flacara	-0.03	+1.61	+7.84	-1.6	+1.91	+5.78	-0.34	+1.69	+6.52
Flacara x Rufina	+1.0	+0.27	+2.24	+0.67	+0.85	+1.11	+0.95	+0.41	+1.82

CONCLUSIONS

The analysis of the growth characteristics of the radicle, stem and seedling of the tomato F_1 parents and hybrids at different temperature levels highlighted the differentiated nature of the reaction of the genotypes under study. In the most cases stressful temperatures produced significant inhibition of tomato growth organs.

The manifestation of significant differences in the characters analyzed in most of the reciprocal F_1 hybrids both in the control variant (25°C) and in the variants with stressful temperatures (40°, 42°C) demonstrates the involvement of the parental factor in the reaction and the formation of the phenotype of the growth characters at different temperature levels. The maternal effect was more pronounced at the temperature of 42°C than under optimal conditions, which indicates the need to take into account the resistance of parental forms when making decisions about the orientation of crosses of hybridization components in order to reduce the effects of stressful temperatures on tomato growth organs in early ontogeny.

According to the degree and orientation of dominance of growth characters in reciprocal F_1 tomato hybrids at different temperatures, the interaction of maternal and paternal genes is strongly influenced by the thermal factor. It was established that in the F_1 L 10B x Rufina and F_1 Rufina x Flacara combinations, the evaluated characters showed, under stressful conditions, overdominance in relation to the best parent, which indicates that in these conditions, the parental entity intensifies the influence of the recessive alleles on the dominant, involved in the control of growth characters of tomato plants.

Through cluster analysis (*k*-means) tomato parents and F_1 hybrids were identified - Dolgonosic, Desteptarea, F_1Vrojainii x Flacara, F_1 Desteptarea x Flacara, F_1 Rufina x Flacara with high resistance to temperature 42°C, which provides opportunities for their use in breeding programs as reliable sources of resilience.

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SEED DORMANCY IN CANCER BUSH (*SUTHERLANDIA FRUTESCENS*): A MAJOR HINDRANCE TO PRODUCTION

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Abstract

Cancer bush, Sutherlandia frutescens is a Southern African indigenous plant harvested for its medicinal properties against several human illnesses. One major challenge in the sustainable development and cultivation of medicinal plants is seed dormancy that prevents the seeds from germination even when exposed to favourable conditions or when sown in the field. In this study, the effect of various chemical (H_2SO), mechanical scarification, physical (hot-water, sodium chloride and cold-water soaking) and biological (Trichoderma harzianum) methods of breaking dormancy were tested. Among all other treatments, hot water was found to be moderately effective in breaking dormancy resulting in 48% seed germination, which is still below the minimum recommended standard germination percentage of 80%. However, the mechanical scarification was the most effective method, resulting in germination percentages of 100%. The other seed treatment methods resulted in less than 10% germination. In conclusion, cancer bush seeds exhibited physical dormancy and the mechanical scarification method is recommended for increased seed germination and germination speed of cancer bush, thus good for field establishment and uniform plant population production.

***Key words**: cancer bush, germination, medicinal plants, seed dormancy, seed priming.*

INTRODUCTION

Cancer bush (*Sutherlandia frutescens* (L.) R. Br.) is one amongst well-known medicinal plants indigenous to Southern Africa (Fernandes et al., 2004) and it is broadly distributed in South Africa, Namibia and Botswana (Prinsloo & Street, 2012). In South Africa, it occurs in the drier parts of Eastern Cape, Northern Cape, KZN, and Mpumalanga Provinces, although it is more abundant in the Western and Northern Cape provinces (Aboyade et al., 2013). Cancer bush is so called because of its medicinal use originating from the Khoi-San and Cape Dutch people against internal cancer as reported by Prinsloo and Street (2012). It also has other potential medicinal uses such treating diabetes, HIV/AIDS symptoms, signs of anxiety, and wound healing (Mills et al., 2005; Van Wyk et al., 2008; Fu et al., 2009).

Cancer bush is one of the many medicinal plants recommended for conservation action by the South African government because of extinction threats, owing to overharvesting and habitat destruction of natural populations (Raimondo et al., 2009 cited in SANBI, 2010-2012). According to Raghu et al. (2018), merely 10% of medicinal plants are cultivated, indicating that harvesting of natural stocks for their benefit are more common. Bringing these species to cultivation (especially the identified endangered species) represent a viable alternative solution to unlimited supply and may also help in sustaining their availability for future generations (Xego, Kambizi & Nchu, 2016). However, there are challenges to cultivation and only a few studies have investigated that, opening a knowledge gap that is a limiting factor to successful commercialization (Nwafor, 2020). Farahani, Hajiberat and Hajiberat (2014), found seed dormancy to be one of the challenges that prevent successful development, mass cultivation and adoption of medicinal plants, as viable seeds remain in the soil for a very long time without germinating, even when exposed to favourable conditions or sown in the field. Seed dormancy acts as a plant establishment delaying mechanism, inhibiting germination leading to uneven crop stand in the field, making it difficult to plant simultaneously and properly maintain plant population (Yildiz, 2018).

Several dormancies that could potentially influence production have been identified by Baskin and Baskin (2000; 2004), and these include exogenous, endogenous and combinational dormancy. Germination-promoting stimuli such as scarification and others have been found to assist seeds in breaking their dormancy (Bentsink, Koornneef, & Hilhorst, 2002). Their use may help in improving seed germination and better the performance in cultivation of medicinal plants. The aim of the study was to use various dormancy breaking techniques to determine the mechanism of dormancy exhibited by the plant and their effect in improving germination of cancer bush.

MATERIALS AND METHODS

Description of study site and preparation of materials

The experiment was conducted at the University of Mpumalanga, (Mbombela campus), South Africa (25º27′06. 18″S 30º58′5.21″E) under laboratory conditions, using viable cancer bush seeds. The seeds were first sterilized in 1% NaOCl for 5min and subsequently rinsed five times with sterile distilled water before applying the different dormancy-breaking treatments (Farahani et al., 2014).

Experimental treatments and layout

Six experiments were conducted to determine the mechanism of dormancy in *Sutherlandia frutescens*: namely the mechanical scarification, acid scarification, hot and cold-water soaking, soaking with NaCl and with *Trichoderma harzianum.* The experiments were laid out in a completely randomized design (CRD) with three replications for each experiment and ten seeds per replication.

Mechanical scarification experiment

Seeds were scarified for 10s opposite the micropyle following the procedure described by Arowosegbe (2016).

Acid scarification experiment

Acid scarification was done under a laboratory fume hood following a procedure by Dada et al. (2019), using three different concentrations of H_2SO_4, 40, 60 and 100% for 2, 4, and 6 min. The seeds were then rinsed thoroughly using sterilized distilled water to terminate the chemical reactions.

Physical treatments

Hot water soaking

Hot water treatment was done by separately soaking seeds in hot water using an EcoBath at 60, 80 and 100°C for 2, 4 and 6 min (Dada et al., 2016). After the hot water bath, seeds were removed and allowed to cool down for 10 min.

Cold water soaking

Seeds were soaked separately in beakers containing distilled water at room temperature for 0, 24, 48, 72 and 96 h (Arowosegbe, 2016).

Soaking with sodium chloride (NaCl)

Seeds were separately soaked in four different levels of salt: 0, 2, 4 and 6 g L^{-1} for 24, 48, 72 and 96 h. All seeds from NaCl treatment were rinsed with double distilled sterile water to remove the salt prior to culturing.

Biological priming

Soaking with Trichoderma harzianum

Seeds were primed using *Trichodema*, 1x 10^7 cfu/g live cells (Dalil, 2014). Firstly, the fungi were grown on a potato dextrose agar (PDA) and incubated at 25 °C for 3 days. To prepare the inoculum, a few drops of water was added onto the plate while a hockey stick was used to detach spores from mycelia. The liquid containing the pores was then collected from the plate. Spore count was done using a haemocytometer and seeds were soaked in water with the *Trichoderma* spores for 3, 6, 12 and 24 h. After soaking, seeds were allowed to air dry under the laboratory laminar flow and then transferred into a growth chamber with standard conditions set at 25 °C.

After all the treatments were applied, seeds were then transferred into a 9 cm diameter sterilized Petri dish containing one-layer Whatman filter paper moistened with 5 ml distilled water daily (Tavili et al., 2010). Seeds were kept in a growth chamber with standard temperature set at 25 °C. Untreated seeds in each treatment were used as a control.

Data collection

Seed were considered to have germinated when the radicle had grown 2 mm beyond the seed coat (Farahani et al., 2014). Germination was recorded in 24-hour intervals for 14 days or until no further germination occurred. Germination-related variables such as germination percentage (GP), mean germination time (MGT), mean daily germination (MDG), germination speed (GS), plumule length (PL) and radicle length (RL) were computed upon termination of the experiments using formulas below as described by Arowosegbe (2016).

The data collected was first tested for normality using Shapiro-Wilk test and then analysed using Statistix 10 software.

Treatment means were separated using LSD-test at 5% probability level. For the completely discrete data (obtained from mechanical scarification) Mann-Whitney U test (non-parametric test) was used to get the actual means rather than the transformed means.

GP % = [(G/N) *100]
Where G = Total number of germinated seeds
N = Total number of seeds in petri dish

GS = (10**n*1) + (9*n2) + (8**n*3) +… (1**n*14)
Where *n*1, *n*2, *n*3...*n*14 are the number of germinated seeds of the 1st, 2nd and 3rd day and following days until the 14th day.

MGT = Σ*f.x*/ Σ*f*
f = is the number of germinated seeds of day *x*

MDG = Total number of germinated seeds/ total number of days

RESULTS AND DISCUSSIONS

All measured variables were not normally distributed ($P \geq 0.05$) as presented by Shapiro-Wilk normality test, hence, were transformed accordingly.

Effect of treatments on germination variables of *Sutherlandia frutescens*

The cold water, *T. harzianum* and sodium chloride treatments had no significant effect on germination, except for the mechanical scarification, acid scarification and sulfuric acid.

Mechanical scarification

The treatments had significant effect ($P \leq 0.05$) on germination percentage, germination speed, mean germination time, mean daily germination, radicle and plumule length. Relative to the unscarified seeds, scarification treatment had significant higher germination percentage, contributing 100% (Table 1; Figure 1).

Figure 1. Cancer bush seeds

The untreated seeds had the lowest germination of 0%. The highest radicle and plumule length were measured when seeds were scarified with sandpaper compared to untreated seeds which recorded 0mm for both the plumule and radicle length.

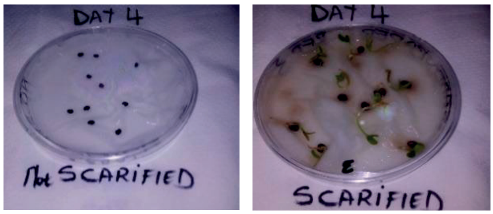

Figure 2. Cancer bush showing 100% germination within four days of culture

Table 1: Effect of mechanical scarification on germination variables of cancer bush (*Sutherlandia frutescens*)

Scarification	GP %	GS	MGT	MDG	RL (mm)	PL (mm)
Scarified seeds	100	23.86	44.58	1.25	13.93	18.73
Un-scarified seeds	0	0	0	0	0	0
U-stat	0	0	0	0	0	0
U-critical $_{0.05}$	0	0	0	0	316	316

Statistically different at $P \leq 0.05$ level according to Mann-Whitney U test (non-parametric test).

Sulfuric acid (H_2SO_4)

The results showed no significant interaction effect between concentration and duration of exposures. However, H_2SO_4 to some extent, was able to break seed dormancy even though it was not effective enough in improving seed germination variables. Seed exposure to 40% H_2SO_4 gave significantly higher GP, MGT, MDG, RL and PL and were significantly different from 60% and 100%, except for MGT at 100% (Table 2). Seed exposure to 60% recorded the lowest in all the variables and were not significantly different from 100%. The results show that the treatment with highest GP also had the highest MGT, MDG, radicle and plumule length and vice versa.

Hot water treatment

Exposing seeds for 4 minutes significantly recorded the highest germination percentage relative to 2 and 6 minutes (Table 3). At 6 minutes there were significantly lower GP, GS and MDG, although 6 minutes was not different from 2 minutes. The results show that the treatment with highest GP also had the highest GS and MDG, vice versa.

Exposing seeds to hot water at 80°C significantly improved GP, GS and MDG, relative to 60 and 100°C which had significantly less effect in all the measured variables (Table 4). Both 60°C and 100°C had lower germination effects and were both similar.

Although there were no significant interaction effects between temperature and time for GP, GS and MDG, a significant interaction effect was observed for MGT, RL and PL (Table 5). The 80°C treatment for 2 minutes had significantly higher MGT, RL and PL, relative to all the other treatments, 60°C for 2, 4 and 6 min, and 80°C for 4 and 6 min.

Table 2: Effect of H_2SO_4 on germination variables of cancer bush (*Sutherlandia frutescens*)

Conc[1] (%)	GP (%)	MGT (days)	MDG	RL (mm)	PL (mm)
40	0.35^a(13.3)	0.62^a(4.0)	0.07^a(0.2)	0.20^a(2.6)	0.23^a(4.5)
60	0.09^b(3.3)	0.19^b(1.4)	0.02^b(0.0)	0.03^b(0.3)	0.05^b(0.8)
100	0.16^b(5.6)	0.32^{ab}(1.9)	0.03^b(0.1)	0.06^b(0.9)	0.06^b(1.1)
F-value	5.61	4.01	4.71	6.66	6.92
P-value	0.0142**	0.0388	0.0247	0.0015**	0.0012**
$LSD_{0.05}$	0.1692	0.3290	0.0353	0.0940	0.1107

[X]Column means followed by same letter(s) are not significantly different at P ≥0.05 according to LSD All-pairwise comparisons. Values in brackets are untransformed means. **Highly significant (P ≤ 0.01), *Significant (P ≤ 0.05). [1]Conc = Concentration

Table 3: Effect of hot water treatment on germination traits of cancer bush (*Sutherlandia frutescens*)

Time (minutes)	GP (%)	GS	MDG
2	21.11^b(21.1)	0.38^b(1.8)	0.09^b(0.3)
4	34.44^a(34.4)	0.62^a(2.0)	0.15^a(0.4)
6	16.67^b(16.7)	0.31^b(1.4)	0.07^b(0.2)
F-value	5.80	7.98	7.23
P-value	0.0128**	0.0040**	0.0058**
$LSD_{0.05}$	11.519	0.1694	0.0457

[X]Column means followed by same letter(s) are not significantly different at P ≥ 0.05 according to LSD All-pairwise comparisons. Values in brackets are untransformed means; **Highly significant (P ≤ 0.01) *Significant (P ≤ 0.05).

Table 4: Effect of hot water treatment on germination traits of cancer bush (*Sutherlandia frutescens*)

[1]Temp (°C)	GP (%)	GS	MDG
60	12.22^b(12.2)	0.29^b(1.2)	0.06^b(0.2)
80	47.78^a(47.8)	0.76^a(3.5)	0.20^a(0.6)
100	12.22^b(12.2)	0.26^b(0.5)	0.06^b(0.2)
F-value	28.54	24.78	29.54
P-value	0.0000**	0.0000**	0.0000**
$LSD_{0.05}$	11.519	0.1694	0.0457

[X]Column means followed by same letter(s) are not significantly different at P ≥0.05 according to LSD All-pairwise comparisons. Values in brackets are untransformed means; **Highly significant (P ≤ 0.01), *Significant (P ≤ 0.05). [1]Temp = Temperature

Table 5: Effect of hot water treatment on germination traits of cancer bush (*Sutherlandia frutescens*)

Time (min)			
MGT (days)			RL (mm)
6	2	4	6
0.54^{bc} (3.8)	0.03^{e}(0.3)	0.38^{ab}(10.3)	0.13^{de} (2.2)
1.12^{a}(12.9)	0.84^{a}(14.4)	0.57^{bc} (7.1)	0.64^{ab} (10.2)
0.00^{d}(0.0)	0.14^{de} (1.33)	0.29^{de} (3.0)	0.03^{e}(0.3)
3.66			2.97
0.0267			0.0201
0.4453			0.2598

^{X}Column means followed by same letter(s) are not significantly different at $P \geq 0.05$ according to LSD All-pairwise comparisons. Values in brackets are untransformed means; **Highly significant ($P \leq 0.01$), *Significant ($P \leq 0.05$).
[1]Temp = Temperature

Seed germination is the most important aspect of plant production and nutrition worldwide (Esan, Ayanbamiji & Abodunri, 2021). A wide understanding of the physiological processes in seeds is significant for crop stand establishment at the field. This study investigated the mechanism of dormancy and the effectiveness of different dormancy breaking treatments in cancer bush seeds which included cold water soaking, hot water, H_2SO_4 and sandpaper scarification, soaking with NaCl and *T. harzianum*. The results shown some variation in cancer bush response when treated with the different dormancy treatments.

Reports indicates that most seeds belonging to the family Fabaceae possess physical dormancy which prevents water and oxygen permeability thus delaying seed germination in such species (Ali et al., 2011). The results obtained from this study also confirm that cancer bush from the same family possess a physical dormancy which was broken by the different treatments, and as the results from untreated seeds was relatively low. This supports even more of the findings by Esan et al. (2021) who found that seeds of wild plant species including cancer bush are dormant relative to the cultivated plant species. Dormant seeds are alive but fail to germinate under conditions that are favourable for non-dormant seeds of the same species (Larson, 2002).

The results of this study revealed that mechanical scarification effectively improved seed germination relative to all other seed treatment methods. Other studies on leguminous seeds or seeds belonging to the Fabaceae family show that mechanical scarification is a very effective method for breaking dormancy of such species and improve the germination (Patane & Gresta, 2006). In species such as *Helianthemum* occurring in arid and semi-arid environments, the hand scarification of seeds was able to significantly improve germination (Pérez-García & Gonzalez-Benito, 2006). Patane and Gresta (2006); Travlos, Economou and Karamanos (2007) both reported that mechanical scarification of seeds is effective for breaking dormancy of leguminous seeds that are native in arid and semi-arid conditions and thus improving seed germination. The ability of the hand scarification method to improve germination is evidence that cancer bush seeds exhibit exogenous dormancy imposed by the hard seed coat.

The physical dormancy that many leguminous seeds exhibit can be broken or eliminated by exposing seeds to concentrated acids such as sulphuric acid (Nadjafi et al., 2006), and its use in breaking dormancy vary depending on the plant species (Uzun & Aydin, 2004). Studies that were conducted on other seeds of the same family, *Cassia occidentalis, C. obtusifolia, Indigofera astragalina, I. tinctoria, I. senegalensis, Tephrosia purpurea* and *Sesbania pachycarpa* (Sy, Grouzis & Danthu, 2001), *Parkia biglobosa* (J acq. Benth) (Aliero, 2003), *Astragalus hamosus* and *Medicago orbicularis* (Patane & Gresta, 2006), *Tylosema esculentum* (Buech) L. Schreib (Travlos, 2007), *Senna alata* (L.) Roxb. (Arowosegbe, 2016), *Senna alata* (Esan et al., 2021) revealed that immersion of seeds to concentrated H_2SO_4 resulted in improved final germination percentage of the dormant seed. The mechanism that led to improving the germination of the seed could have been the ability of the acid to break the hardened seed coat allowing water permeability and oxygen exchange (Ali et al., 2011). In this study, the use of H_2SO_4 to some extent, was able to break seed dormancy giving the maximum germination of 13.3% which is still not effective enough in improving seed germination, yet it was significantly better than the untreated seeds. The results showed that increasing the concentration beyond 40 to 100% and the duration of exposure resulted very low final germination percentages to lower final germination percentages, lower than 13.3%. The factors that could have led to the decrease in germination percentage might be the loss of seed viability due to damaged embryo after seed immersion in H_2SO_4 (Pipinis et al., 2017). According to Pipinis et al. (2017), when the duration of acid scarification is prolonged, a significantly reduction in germination percentage will occur due to loss of seed viability. A response observed by Bhardwaj et al. (2016) is that any increase or decrease in the duration of *R. webbianum, C. carvi, S. lappa* and *B. persicum* seed immersion in the acid led to a significant reduction in germination because of damaging effects of acid on the embryo's vital parts.

Seed soaking in water at room temperature, irrespective of the duration of soaking, had no significant effect on the final germination percentage, however, when seeds were soaked in water at elevated temperatures it resulted in significantly higher final germination percentage. In this study it was observed that the treatment of 80°C, irrespective of time yielded the highest germination and anything beyond that resulted in subsequent reduction in final germination percentage. Same with the time at 4min, irrespective of the temperature improved germination. The results agree to the findings of Arowesebe (2016) who reported that increasing the water temperature improved germination. Aliero (2003) and Muhammad (2018) both reported hot water soaking act on the seed coat walls causing it to rapture and allow water to penetrate the tissues inside the seed causing physiological changes and improve germination. However, this was different when seeds of *A. muricata* were exposed to hot water baths which had no effect on germination percentage, irrespective of the duration of exposure (Dada et al., 2019). Pre-sowing treatments that produced the highest germination percentage include scarification, and hot water which also resulted in increased germination speed. All other priming methods including the cold-water soaking, NaCl, and *Trichoderma harzianum* resulted in a slightly higher final germination percentage compared to the control, however, they were not effective in improving the germination of cancer bush seeds.

CONCLUSIONS

Among all the various pre-sowing treatments used, hot water at 80°C, irrespective of the duration of exposure improved seed germination and were effective when compared to other seed treatment methods. However, mechanical scarification was able to completely break seed dormancy resulting in the best of all methods with a 100% germination. Medicinal plants are able to survive environmental conditions that are harsh or unfavourable, such as heat stress or mechanical damage which occurs in their natural habitat, and this is shown by the ability of the seed to germinate when exposed to elevated water temperatures or scarified with a sandpaper. Their ability to withstand such condition is of ecologically importance to them, such that it allow the seeds to accumulate into the soil increasing chances that some of the seeds will germinate producing new populations. However, this survival strategy is not effective when fast and constant seed germination is required, it is a limiting factor when the crop is to be cultivated. Based on the results it is suggested that cancer bush has physical dormancy caused by impervious seed coat. The two scarification treatments will therefore be good and recommended for good crop field establishment and obtaining uniform plant population. All other techniques are not effective practice as they resulted in seed germination of less than 20%.

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EFFECT OF INTERCROPPING ON THE GROWTH AND YIELD OF CABBAGE

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Abstract

The aim of the research was to test how different plant species affect the growth and yield of cabbage when they are intercropped. The experiment was carried out in the experimental field of the University of Forestry - Sofia, in 2022. Five combinations were selected: cabbage+leek, cabbage+green beans, cabbage+dill, cabbage+tagetes, cabbage+"Weleda" seed mix, which were compared cabbage as sole crop. The seedlings of cabbage were planted in the period 25-29.07.2022 on the block method with four replications. The other crops were sown or planted at the same period. Compared to intercropping, sole crop cabbage has a smaller height and diameter of the product part. With the highest weight per cabbage is the combination of cabbage + green bean, while the lowest is the cabbage + Weleda seed mix, followed by cabbage + dill.

***Key words**: biometry, cabbage, green bean, intercropping, yield.*

INTRODUCTION

Growing two or more plants together at the same time is called intercropping (Gliessman, 1985; Singh et al., 2013; Maitra and Gitari, 2020). Several varieties of intercropping have been described: row intercropping, strip intercropping, mixed intercropping, and relay intercropping (Gliessman, 1985; Gallaher, 2009; Mousavi and Eskandari, 2011).

The increase in crop biodiversity, thanks to the mixed cultivation of two or more crops, makes intercropping a suitable practice for organic production (Lithourgidis et al., 2011; Yildirim and Ekinci, 2017; Maitra and Gitari, 2020; Mala et al., 2020).

Hailu (2015), in his review article, summarizes the advantages of intercropping, namely that it positively affects the microclimate in crops as well as reduces pest development.

As for the development of the plants and the yields obtained, the conclusions of the scientists are different. In some of the experiments, plants in intercropping developed better and obtained better yields compared to the sole cultivation of the crops (Hailu, 2015; Yildirim and Ekinci, 2017; Maitra et al., 2021), while in others there was no difference between the two systems or poorer results were obtained (Hailu, 2015; Maitra et al., 2021).

Cabbage is one of the main vegetable crops of the Brassicaceae family. In intercropping, it can be grown as a main crop or as a companion crop due to its large rosette of leaves covering a large part of the area. Neupane al. (2021) selected cabbage and mustard as companion crops in growing potatoes and testing different planting schemes. They found that in the intercropping of potatoes and cabbage, with and without the participation of mustard in the plantations, an increase in the height of the potato and cabbage plants was obtained, resulting in a higher yield of both crops, as well as an improvement in the cabbage cover. When growing a main crop of maize with an intercrop of cabbage, the highest yield of both crops was obtained when the cabbage was grown in between the maize rows (Khanum et al., 2019).

When cabbage is the main crop, usually inter-row combinations are sown with companion species, and the results can be different. For example, Guvenc and Yildirim (2006) found that growing radishes in between cabbage rows had an adverse effect on cabbage yield. On the other hand, Kumari et al. (2021) proved that when growing cabbage in combination with radishes, good results were obtained both in terms of yield in both crops and in terms of biometric indicators of cabbage heads (height and diameter, weight, and total soluble sugars).

Mixed cultivation of cabbage with legumes also shows different results. A combination of green beans with spring forms of cabbage produced the highest yields of both crops (Shanmugam et al., 2022). In the intercropping of cabbage with peas, although the yield of cabbage is second to that of solely cultivated cabbage, the equivalent yield is higher because of the higher yield of the companion crop, the green pea (Choudhuri and Jana, 2012). On the other hand, Bavec et al. (2011) recorded low yields of cabbage in combination with bush beans.
High yields were also obtained in the mixed cultivation of cabbage with leafy vegetables: mixed cultivation of cabbage with leaf lettuce or cos lettuce (Guvenc and Yildirim, 2006; Bavec et al., 2011); good yields were also obtained in mixed cultivation with spinach because of the three realized harvests of spinach (Kumari et al., 2021).
The paper presents the results of a field intercropping experiment with a main crop of cabbage and five companion crops, which is the first year of the PhD thesis experiment.

MATERIALS AND METHODS

The experiment was carried out in 2022 at the experimental field "Vrazhdebna" (42° 7′ N, 23° 43′ E, and 552 m above sea level) at the Faculty of Agronomy of the University of Forestry on fluvisol.
The climate is moderately continental, and the weather conditions during the experimental period were favorable for plant development (Figures 1 and 2).
The main crop is cabbage (*Brassica oleracea* L. var. *capitata*), cv. "Balkan", a traditional cultivar for Bulgaria, intended for late field production. The accompanying crops are leek (*Allium porrum* L.), cv. "Starozagorski", green bean (*Phaseolus vulgaris* L. var. *nanus*), cv. "Saksa", dill (*Anethum graveolens* L.), cv. "Mesten", French marigold (*Tagetes patula* L.), and a mix of seeds from flowering honey plants of the "Weleda" company (buckwheat, marigold, dill, borage, cornflower, and phacelia). Six variants have been developed: V1: solely grown cabbage as the control; V2: cabbage + leeks; V3: cabbage + green bean; V4: cabbage + dill; V5: cabbage + French marigold; V6: cabbage + seed mix.
The experiment was carried out in the last ten days of July, according to the block method, in four replications, with the size of the experimental plots being 3.5 m x 5 m, and the recultivated plots - 2.1 m x 4 m. Cabbage, leeks, and French marigold are planted as seedlings, while beans, dill, and seed mix are sown directly. Cabbage was planted according to the standard scheme of 0.70 m x 0.50 m on previously prepared furrows. Between the rows of cabbage were planted: leeks, according to the scheme 0.70 m x 0.20 m (V2), and French marigolds, according to the scheme 0.70 m x 0.25 m (V5), and were sown: green beans, according to a scheme of 0.70 m x 0.30 m (V3). Dill (V4), and a mix of seeds (V6), were sown thinly between cabbage rows.
The agrotechnical practices during the growing season (sprinkler irrigation and cultivation) were the same for all variants.
During harvesting, measurements of the height, diameter, and weight of the product parts of the cabbage were taken, with the measurements for an average of 10 heads of variants and replications. Samples were taken from the production, including variants and replications, to establish the dry matter content. Yields are calculated per ton per hectare based on average head weight.
The dry weight of the cabbage heads was determined by taking average samples by variant and replication (from 4 heads of each variant). The samples were taken from the edible parts of the head (leaves) and from the internal core of head.
The collected data were analyzed by ANOVA.

RESULTS AND DISCUSSIONS

The meteorological conditions during the vegetation period of the plants are typical for the area. The highest average daily temperatures are recorded at the end of July (26 and 27.06. - 28°C), during the early stages of plant development. In August, the average daily temperature is up to 25°C, then a warm autumn follows, and only in the third ten days of November do the temperatures fall around or below 5°C (Figure 1)
Precipitation during the experimental period was distributed in August, September, and October, with higher amounts recorded mostly

in September (147.5 mm), while amounts in August and October were almost the same, 61.3 mm for August and 58.7 mm for September, respectively (Figure 2).
The largest amount of rain (40 mm) fell at the end of September (the evening of the 28-th, 2022), started by a hailstorm that also caused a drop in temperatures (Figures 1 and 2). Regardless of the rainfall that fell and its relatively good distribution in time, in terms of quantity, most of them were insufficient, and it was necessary to carry out sprinkler irrigation every week..

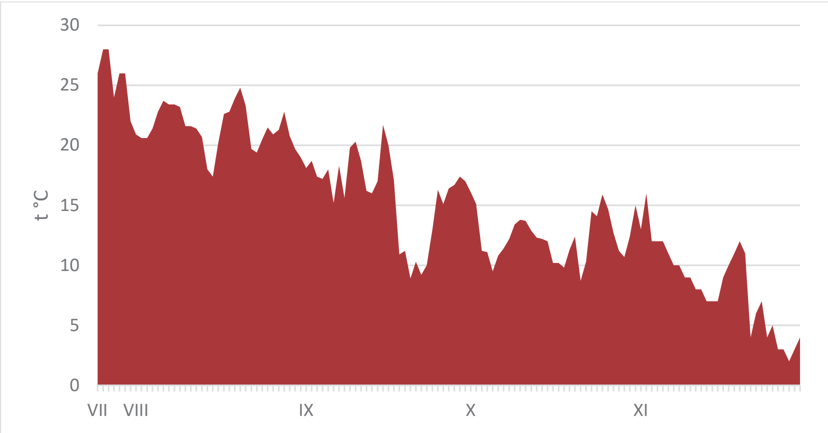

Figure 1. Average daily temperature during the experimental period at Vrazhdebna experimental field, 2022

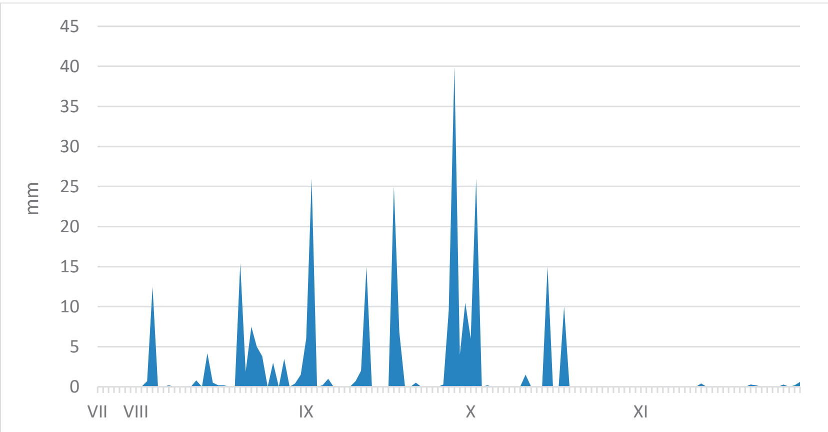

Figure 2. Precipitation during the experimental period at Vrazhdebna experimental field, 2022

The first harvest occurred on October 25, 2022. The harvests continued until the end of November. Simultaneously with the harvesting of the produce, the height and diameter of the product part of the cabbage were measured.
In general, in all variants with mixed cultivation, the values of the height of the cabbage heads were greater compared to the control variant (sole cultivation).
The highest height was measured in the cabbage + French marigold combination (18.24 cm) and the smallest in the cabbage + seed mix combination (16.85 cm). With a slight difference (16.53 cm) from the last combination is the control variant (Figure 3)
When arranging the combinations in descending, they are ordered as follows: cabbage + French marigold > cabbage + dill = cabbage +

green beans > cabbage + leeks > cabbage + seed mix > and sole cabbage (Figure 3)

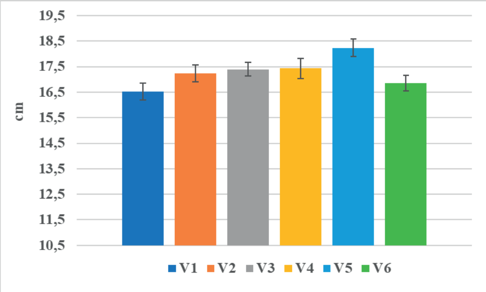

Figure 3. High of cabbage head, cm (means ± SEM)

According to the indicator of cabbage head diameter, again the variants with intercropping exceeded the control with minimum differences (1.15 cm).

However, again the largest diameter was the variant cabbage + French marigold (21.35 cm) and the smallest was the control (20.2 cm), but when arranging the variants in descending order to assess their influence, the order was different: cabbage + French marigold > cabbage + green beans > cabbage + leeks = cabbage + seed mix > cabbage + dill > and sole cabbage (Figure 4)

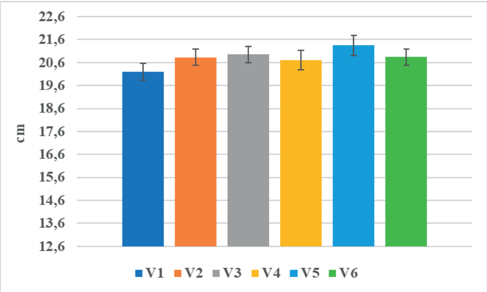

Figure 4. Diameter of cabbage head, cm (means ± SEM)

Regardless of the small differences, the influence of mixed cultivation on the height and diameter of the cabbage heads is outlined, and in both indicators, the cabbage + French marigold combination exceeds the others.

According to Neikov (2003) the limits of both indicators of the size of the cabbage head (height and diameter) for this cultivar, are 16-25 cm height and 15-20 cm diameter. Comparing these values with the obtained data, it can be seen that, according to the diameter indicator, all variants are within and above the limits, with the control being close to the standard and the other variants being above.

The results obtained at this stage for both indicators are in contrast to the data from Guvenc and Yildirim (2006), where the sole cabbage has a larger diameter and height of the cabbage heads compared to the intercropping options.

Although the intercropping of cabbage with green beans (V3) is slightly behind the previous indicators of intercropping with French marigold (V5), according to the indicator of average fresh weight per head, it is first (Figure 5).

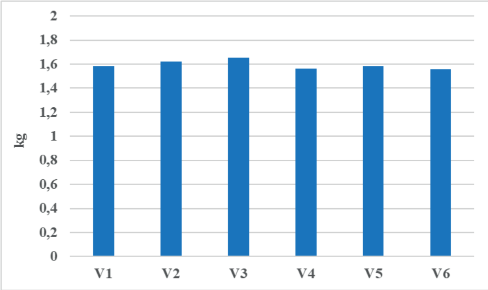

Figure 5. Fresh weight of cabbage head, kg/plant

The average fresh weight of one head of cabbage for variant cabbage + green bean is 1.652 kg, and the lowest weight (1.557 kg) is for the variant cabbage + seed mix (Figure 5).

There are no significant differences between the variants, which confirm the result obtained from Guvenc and Yildirim (2006).

Although the differences in the dry matter content, of the edible part of the cabbage head, are not significant, two variants have a higher percentage: variant 2: intercropping cabbage + leeks (8.2%); and variant 4: intercropping cabbage + fennel (8.1%). The intercropping with green beans has the lowest dry weight (7.6%) of the edible part (Figure 6).

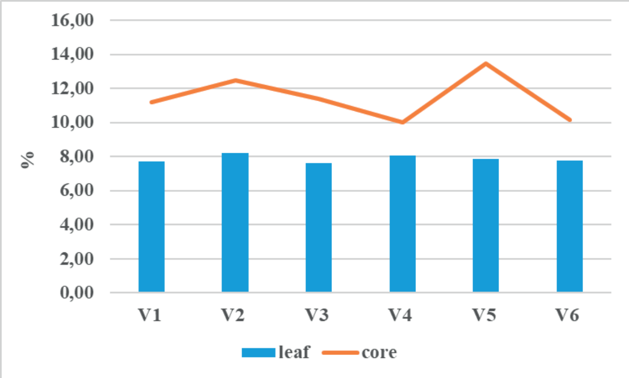

Figure 6. Dry weight of cabbage head, %

The dry matter of the inner core of the cabbage showed different results than the edible part. Cabbage + French marigold intercropping has the highest dry weight percentage of the core (13.5%), followed by variant 2: intercropping cabbage + leeks (12.5%). With the lowest dry weight are: variant 4: intercropping cabbage + fennel (10%) and variant 6: intercropping cabbage + seed mix (10.2%).
Yield is calculated in tons per hectare. The yields of all variants are within the limits of the average yield for the cultivar used, as indicated by Neikov (2003).
Although the differences in yields are small, the variants can be divided into three groups. The first group includes only V3, which has the highest yield (47.6): intercropping cabbage + green beans (Figure 7).

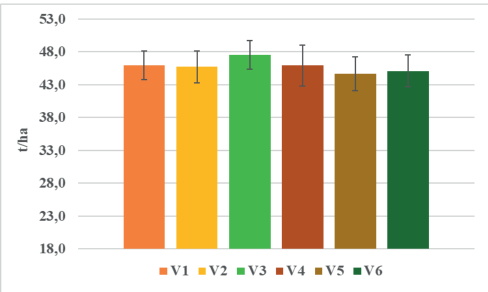

Figure 7. Average yield of cabbage, t/ha (means ± SEM)

In the second group, there are three variants with an average yield of cabbage (45.9-45.7 t/ha): sole cabbage (V1), intercropping cabbage + dill (V4), and intercropping cabbage + leek (V2).
In the third group are the last two variants: variant 6 (45.1 t/ha) intercropping cabbage + seed mix and the variant with the lowest yield (44.7 t/ha) intercropping cabbage + French marigold.
The low yield of variant 5 (intercropping cabbage + French marigold) is contrary to the results obtained by Mrnka et al. (2023), who obtained high yields from intercropping cauliflower with French marigold.

CONCLUSIONS

The results of this stage of the experiment revealed that the option of intercropping with green beans had the most complex effect on the reported indicators of cabbage heads and cabbage yield. This variant exceeded the control variant (sole cabbage) in all indicators.
According to the presented indicators, in comparison to sole cabbage, two other companion crops (leek and fennel) also showed better or comparable results.

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ESTIMATION OF CHLOROPHYLL CONTENT AND DETERMINATION OF CHLOROPHYLL FLUORESCENCE IN BITTER CUCUMBER (*MOMORDICA CHARANTIA* L.) LEAVES UNDER SALINE STRESS CONDITIONS

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Abstract

It has been scientifically proven that one of the physiological processes most affected by salt stress is photosynthesis. In this context, it is important to identify the main mechanisms involved in increasing plant tolerance to salt stress. The photosynthetic intensity is directly related to the productive capacity of plant species, thus increasing the tolerance of plants to saline stress is a priority. The chlorophyll content and chlorophyll fluorescence were studied in two varieties (Rodeo and Brâncuși) and three lines (Line 1, Line 3 and Line 4) of bitter cucumber (Momordica charantia), subjected to salt stress. Following the determinations, a higher concentration of chlorophyll was estimated in the control plants of the Brâncuși variety and Line 4, in contrast to the Rodeo variety, Line 3 and Line 1. In the case of this variety and the two lines, the plants treated with the highest concentration of saline solution prepared by using sodium chloride (NaCl) (200 mM) shows a higher concentration of total chlorophyll content. During the treatments, the fluorescence was higher in the treated plants compared to the control plants.

Key words: *bitter cucumber, salt stress, chlorophyll, fluorescence.*

INTRODUCTION

Bitter cucumber (*Momordica charantia* L.) belongs to the: Class Dycotyledonnae, Order Cucurbitales, Cucurbitaceae Family, *Momordica* genus. Bitter cucumber also known as bitter gourd is a tropical and subtropical plant grown in India, Asia, South America, Caraibbes, South Africa (Agarwal & Shaheen, 2007; Şesan, 2020).

The bitter cucumber was cultivated for the first time in Romania in 1990 when some seeds were supplied from Nepal. It is cultivated and researched in Romania within the Vegetable Development Research Station Buzau where the two varieties and the three lines studied in this paper were obtained (Şesan, 2020).

The plant is well known for its many medicinal properties, including anti-inflammatory; anti-carcinogenic; antitumor; antimicrobial; antibacterial; antifungal; antiviral; and hypoglycemic. The fight against diabetes being the main reason for eating bitter cucumbers (Martin et al., 2004). The fresh juice of *M. charantia* could control blood sugar and insulin levels as well as improve the human digestive system (Busuioc et al., 2020).

In addition to its polyphenol-rich content, this plant contains certain phytocompounds with a significant hypoglycaemic role, such as: charantin, momordicin, momordenol, zeatin, zeinoxanthin amino acids, polypeptides, sometimes being referred to as insulin plant (Ştefan et al., 2022).

Salt stress is one of the main factors that question the future of plant crops (Săulescu et al., 2010). Environmental stress, including salinity, affects almost every aspect of plant physiology, biochemistry and significantly diminishes the yield (Heidari, 2011).

About 20% of the world cultivated lands and about half of all irrigated lands are affected by saline stress (Zhu, 2001).

The high concentrations of salt in the soil, especially sodium chloride, cause osmotic stress by lowering the potential of water in cells and ion stress due to inhibition of metabolic processes.

In response to saline stress, plants accumulate toxic ions in vacuoles. Plant cells must also undergo osmotic adjustment, which is accomplished in many ways, including the production of organic osmolytes such as glycine betaine, proline, some sugars, and polyamines, most of which are synthesized in the chloroplast (Di Martino et al., 2003; Hameed et al., 2021).

One of the main physiological processes affected by excessive soil salinity is photosynthesis. Photosynthesis is the most important physiological process by which green plants synthesize organic substances from CO_2, H_2O and mineral salts in the presence of sunlight and assimilatory pigments, yielding O_2 to the environment (Toma & Jităreanu, 2007).

Reduced photosynthesis due to excessive salinity in the environment is not only attributed to stomatal closure, which leads to reduced intercellular CO_2 concentration, but also to non-stomatal factors such as overproduction of reactive oxygen species (ROS) and depletion of potassium (K^+) within plant cells due to natrium (Na^+) accumulation (Stepien & Kłbus, 2006; Hameed et al., 2021).

The present work aims to test the varieties and lines of bitter cucumber to salt stress by following the content of assimilatory pigments and chlorophyll fluorescence.

MATERIALS AND METHODS

The experience was carried out in the greenhouse of the Research Institute for Agriculture and the Environment (ICAM) located on the territory of the Ferma Vasile Adamachi didactic resort, which belongs to the Ion Ionescu University of Life Sciences from Brad in Iași and in the solariums of the Vegetable Cultivation discipline located on the territory of the didactic resort Vasile Adamachi in 2022.

Two varieties (Brâncuși and Rodeo) and three lines (Line 1, Line 3 and Line 4) of *Momordica charantia* (bitter cucumber) obtained at the Buzău Vegetable Research and Development Station (SCDL) were used for the experiment. To obtain the plants used, the seeds were sterilized with sodium hypochlorite (NaOCl), then went through the imbibition process for 24. The seedlings were obtained in the greenhouse under controlled conditions of temperature and humidity. Seedlings were moved to the greenhouse when they had between two and three leaves. They were planted in 12 liter pots filled with a mixture of Florasol flower soil and Kekkila peat.

The bifactorial experiment was performed in randomized blocks with 3 replications. The two cultivars and three lines of bitter cucumber were subjected to salt stress for a period of 40 days.

Plants were treated with saline solutions of different concentrations (M = untreated control plants; V1 = 100 mM saline; V2 = 200 mM saline). The concentrations applied in the current study were chosen to be approximately the average of the maximum and minimum concentrations found in the literature for bitter cucumber. The plants were subjected to a number of four treatments and the determinations were made 7 days after the application of each treatment.

In the present study, the aim was to determine total chlorophyll content and fluorescence.

Total chlorophyll pigment content was estimated using the Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter, which uses a non-destructive method for evaluating chlorophyll content. The method is based on the determination of the chlorophyll concentration index. The chlorophyll concentration index is defined as the difference in optical density at two wavelengths: 650 nm and 940 nm (Bologa, 2016). 10 determinations were made on the leaves from the base of the plant to the middle and from the tip, in total obtaining a number of 30 determinations per plant.

Fluorescence is the characteristic of a solution of pigments to show a different color depending on how it is viewed. If it is seen transparently, in direct light, it has a green color. If seen in reflection, it is ruby-red. Thanks to this property, chlorophyll transforms the wavelength of light radiation, from a short wavelength to a long wavelength (Jităreanu, 2007).

Fluorometer OS30p+ is an instrument that measures fluorescence parameters in the visible spectrum by observing the wavelength intensity distribution of the emission spectrum during excitation with a certain light stimulus. The measurements were aimed at determining the values of the Fv/Fm ratio in which it represents the maximum quantum yield of photosystem II (PS II), the indicator of the maximum efficiency of excitation energy transfer.

The determinations were made on leaves from the base of the plant, from its middle and from the tip. 10 determinations were made from each area, in total 30 determinations for each plant. To make a correlation between the two studied parameters, the Pearson Test was used as a method of correlation.

RESULTS AND DISCUSSIONS

Compared to the control variants watered exclusively with water, the plants exposed for 40 days for saline stress have recorded higher SPAD values, which denotes that the variants of bitter cucumber are included in the biphasic model proposed by Munns. Due to their resistance to saline stress factors, throughout the treatments the plants remained in the first phase of this model (Munns, 1993).

Seven days after the application of the first treatment, in the case of the treated plants, a higher SPAD value is observed compared to the control plants. According to Figure 1, this phenomenon appears in the case of the two varieties: Brâncuși and Rodeo, but it is also observed in Lines 1 and 3. The only variant where the phenomenon is not visible is Line 4, where in the case of control plants, the average of SPAD value shows 42.616. In the case of plants treated with 100 mM saline solution, the SPAD value is 35.888 and in the case of plants treated with 200 mM saline solution, the average SPAD value is 36.722.

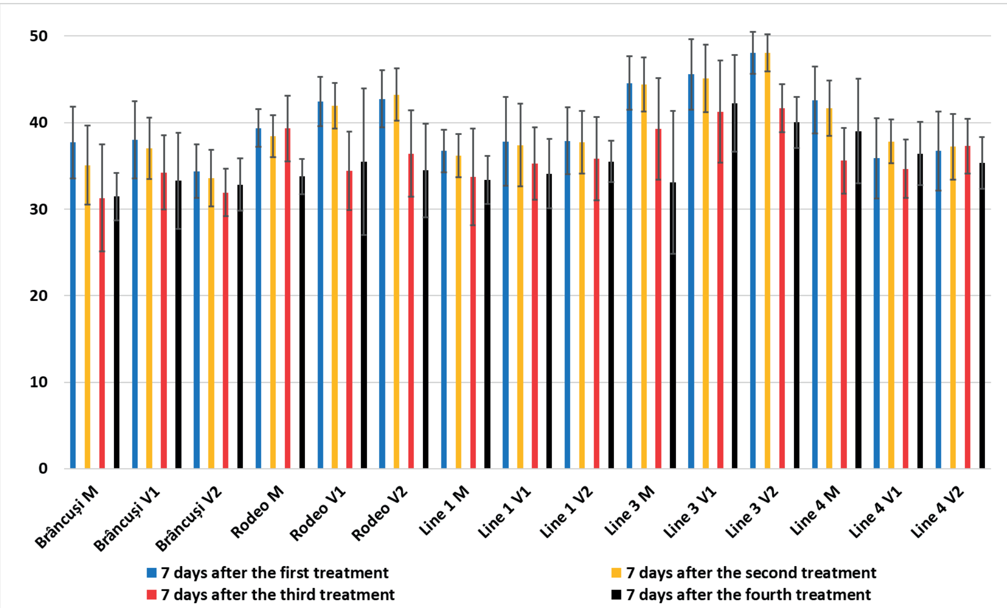

Figure 1. The estimated total chlorophyll content (SPAD values) of *Momordica charantia* L. leaves under saline conditions

After applying treatment number 2, the variants kept the same physiological reaction as in the case of the first treatment. In the case of the Rodeo variety, of Line 1 and Line 3, an increase in the SPAD value was observed, directly proportional to the increase in the concentration of the saline solution applied. In the case of the Brâncuși variety, the highest chlorophyll content is noted in the plants treated with a 100 mM saline solution where the average is 37.011. Control plants have a SPAD value of 35.083 and in the case of plants treated with 200 mM saline solution, the average SPAD value is 33.600. In the case of Line 4, the SPAD value decreases in direct proportion to the concentration of applied saline solutions, thus the highest SPAD value is recorded in control plants and the lowest in plants treated with the highest saline concentration.

Following the determinations made after the application of treatment number 3, the Brâncuși variety, Line 1 and Line 3 presented the same characteristics as in the case of the previous treatment. They show an increase in the SPAD value in the plants treated with the saline solutions compared to the control plants. The Rodeo variety shows a higher SPAD value in the case of the control slopes: 39.333 in contrast to the treated plants. In the case of the treatment with 100 mM, the average SPAD value is 34.422 SPAD units, and in the case of plants treated with 200 mM saline solution, the average SPAD value is 36.400. In the case of Line 4 a difference appears compared to the previous treatments. Plants treated with the highest concentration of saline have the SPAD value: 37.300, control plants have an average SPAD value of 35.616 and plants treated with 100 mM saline have an average photosynthetic pigment of 34.655. After applying treatment 4, it is observed that the Brâncuși variety, Line 1 and Line 3 remain constant. The Rodeo variety changes its pigment content in the control plants, which present the lowest concentration: 33.783. The plants treated with the 100 mM saline solution show the highest amount of chlorophyll: 39.911, and the plants treated with the 200 mM concentration solution show an average of chlorophyll of 34.466 SPAD units. Line 4 returns to the characteristics observed after the first two treatments where the content of chlorophyll decreases proportionally with the increase in the concentrations of saline solutions.

In the specialized literature, I did not find similar studies to be able to compare the results.

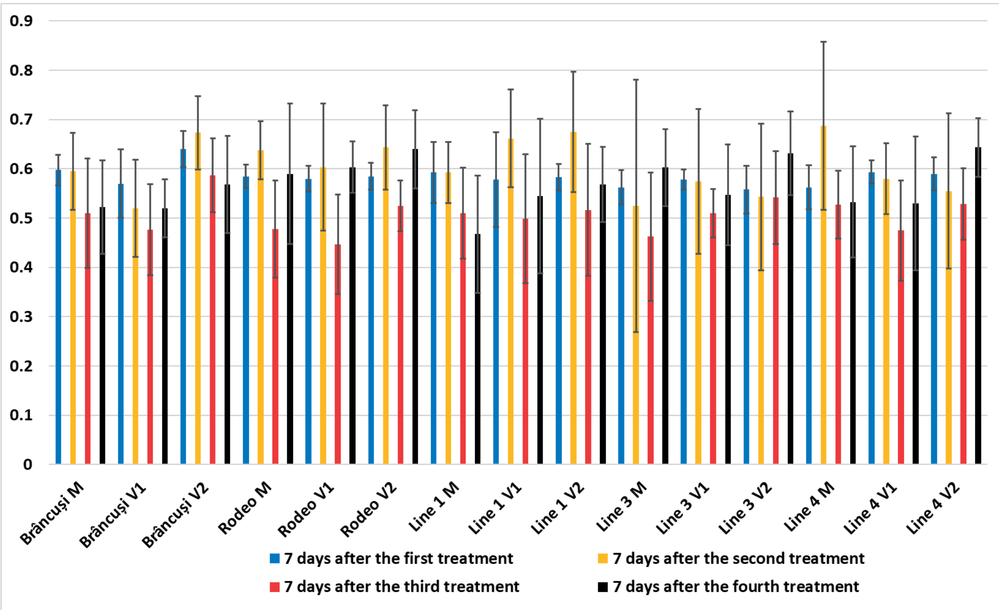

Figure 2. Chlorophyll fluorescence values (Fv/Fm) of *Momordica charantia* L. leaves under saline conditions

Exposure of bitter cucumber plants to saline treatments influenced their ability to capture light. These results correlate with the total content of photosynthetic pigments in leaves which is in the first phase of the biphasic model proposed by (Munns, 1993).

Determinations after the first treatment show a high level of fluorescence in plants treated with saline solutions compared to the control. Among all the variants taken in the fluorescence calculation, the highest fluorescence was recorded in the Brâncuși variety in the plants treated with 200 mM saline solution, where the average of the fluorescence determinations was 0.64 μmol. In the case of the Rodeo variety, no significant differences are observed between the control and the treated variants. In the case of Lines 3 and 4, the highest fluorescence values were recorded in plants treated with 100 mM saline solution. The lowest fluorescence value was recorded at Line 3 in the case of plants treated with 200 mM saline solution: 0.558

μmol. In the case of Line 1, the highest fluorescence value is in the case of control plants.
After the second treatment, Brâncuși and Rodeo varieties remained constant. Line 3 showed the highest fluorescence value in plants treated with 100 mM saline where 0.575 μmol was recorded. In the case of Line 4, the fluorescence value decreases proportionally with the increase in the applied saline concentrations, and in the case of Line 1, the values increase directly proportionally with the increase in the concentration of saline solutions.
Following the determinations made after the application of treatment number 3, it is observed that the only variant that remained constant is the Brâncuși variety. The variety Rodeo, Line 1, Line 3 and Line 4 show the highest fluorescence value in the case of plants treated with the highest concentration of saline. Line 3 shows an increase in fluorescence values directly proportional to the increase in saline solutions applied.
After treatment 4, it was observed that the varieties Brâncuși and Rodeo but also Lines 1 and 4 show an increase in fluorescence values directly proportional to the increase in the concentration of the saline solutions applied. Line 3 shows a value of the control plants of 0.602 μmol compared to the value of the plants treated with 200 mM saline solution, where the value of 0.631 μmol was recorded, the lowest value being recorded in the plants treated with 100 mM concentration solution.
The Pearson test was determined to evaluate the impact of the treatment with saline solutions, on the degree of connection between the variables that define a series of physiological indicators involved in the response to salt stress, in the bitter cucumber (*Momordica charantia*).
In order to identify the behavior of the bitter cucumber to the abiotic stress factor applied during the successive treatments, determinations of the total chlorophyll content and fluorescence were performed for each treatment, which were then subjected to the Pearson test.
In the case of the 4 determinations made 7 days after the application of each treatment with saline solution, the Pearson test shows that increasing abiotic stress determined a different degree of association between the analyzed.
The Pearson test shows that the salt stress to which the bitter cucumber plants (*Momordica charantia*) were subjected, determined a different degree of association between the analyzed parameters. The values show that salt stress involves a specific behavior on the part of the plants depending on the phenophase.
In the Biologische Bundesanstalt Bundessortenamt and Chemische Industrie (BBCH) scale, 21 phenophase, according to the Pearson test, after the first applied treatment the correlation between the two variables taken into account was negative, falling between the values -0.5 and -0.75 (according to Table 1), values that assume a moderate to good correlation coefficient (Oancea, 2007).
In the BBCH 51 phenophase, which corresponds to the second treatment, the Pearson test indicates a correlation coefficient between the variables taken into account, between -0.25 and 0.50 (according to Table 1). The value corresponds to a correlation with acceptable degree of association (Oancea, 2007).
Treatment number three corresponds, depending on the bitter cucumber variant, to BBCH 61 or BBCH 62 phenophases. In these phenophases, the Pearson test indicates a correlation coefficient of the two variables between -0.25 and 0.25 (according to Table 1), values that correspond to a correlation weak or null (Oancea, 2007).

Table1. Pearson correlation coefficient between estimated chlorophyll content and chlorophyll fluorescence

BBCH Scale	21	51	61-62	71
Pearson corelation	-0.7173	-0.3069	-0.09857	-0.207368

The last treatment was applied in the BBCH 71 phenophase, which is equivalent to the appearance of the first fruit on the plant. The Pearson test indicates in this phenophase, values between -0.25 and 0.25 (according to Table 1). As with the determinations after the third treatment, the values show little or no correlation (Oancea, 2007).

Following the performance of the Pearson Test, a negative correlation is observed in the case of all the phenophases in which the determinations were made.

During the development of the plants and the phenophases of the BBCH scale, the correlation ratio between the studied parameters decreases. In the first studied phenophase, a good correlation is observed and in the last phenophase, a weak to zero correlation is noted.

CONCLUSIONS

The analysis of SPAD value revealed that the most affected variant of bitter cucumber (*Momordica charantia*) during the period of all four applied treatments was Line 4, which recorded the lowest values compared to the control plants.

The most resistant variant to salt stress during all treatments was Line 3, in which a greater amount of SPAD value of was observed in plants treated with the two salt concentrations compared to control plants.

The interpretation of the data obtained with the Fluorometer OS30p+ showed a good physiological reaction of the plants to salinity.

The most resistant variant of bitter cucumber was the Brâncuși variety where the plants treated with 200 mM saline solution showed a marked difference. Also, they showed a higher fluorescence compared to the control plants during the treatment period.

Following the performance of the Pearson Test, a negative correlation is observed in the case of all the phenophases in which the determinations were made.

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EVALUATION THE SWISS CHARD RESPONSE TO BIOCHAR APPLICATION

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Abstract

A study was made to determine the influence of incorporate carbonized plant residues, as a soil improver, on the growth and development of Swiss chard (Beta vulgaris subsp. vulgaris). A pot experiment was conduct on two soil types: Vertisols and Luvisol soils. Five variants were develop in three replicates, as follow: 1. Control – pure soil without fertilization; 2. Soil + biochar (BC); 3. BC+$N_{200}P_{200}K_{250}$; 4. BC+$N_{400}P_{200}K_{250}$; 5. BC+$N_{600}P_{200}K_{250}$ and the same variants (6, 7, 8, 9, 10) on Luvisol. Irrespective of the different starting supply of nutrients in two soil types, the highest yield of plant mass was recorded in the variant with BC+$N_{200}P_{200}K_{250}$, followed by the variant with BC+$N_{400}P_{200}K_{250}$. The fresh mass recorded on Vertisol significantly exceeds that on Luvisol.

Key words: *Swiss chard, fertilizer, biochar, pot experiment.*

INTRODUCTION

Applying organic residues to the soil is one of the main ways to improve soil fertility. It is also one of the strategies for soil management and part of the "circular economy". In this regard, in recent years there has been a growing interest in the use of safe products obtained through organic waste recycling technologies, such as biochar.

Biochar is a porous, carbon rich material produced by heating organic matter to temperatures between 300°C and 1000°C in an environment with limited or no oxygen (Verheijen et al., 2010).

These organic amendments are reported to mitigate climate change by atmospheric CO_2 sequestration, to improve soil physical (aggregation, density, root penetration) and chemical (pH and CEC) properties, water-holding capacity and nutrient retention, organic matter and nutrient cycling, to stimulate soil microbial, microfauna, and mesofauna communities, to reduce nutrient leaching, and to increase heavy-metal sequestration (Libutti et al., 2021).

Application of BC can positively affect plant nutrient uptake and facilitate the efficient use of fertilizers by keeping them on its surface and preventing their leaching from the soil (Gunarathne et al., 2017).

Leafy vegetables that require minimal processing are of great interest to consumers due to their ease of use. To increase the yield and quality of leafy crops, farmers often apply large amounts of nitrogen fertilizer.

Swiss chard is one of the leafy vegetables suitable for cultivation, through organic fertilization, and it is widely spread in the countries of Central and Western Europe. Swiss chard plants have been used for many years in traditional medicine to treat various diseases and conditions. Many of these medicinal plants are also excellent sources of phytochemicals, many of which contain powerful antioxidants. Nitrogen overdoses can have a negative impact on the environment and the taste qualities of raw and minimally processed vegetables. Studies conducted with Swiss chard show that plant has the ability to accumulate large amounts of nitrates in its leaf mass, which affects its quality (Colla et al., 2018).

Libutti (2020) mace the research on Swiss chard response to biochar and compost application, they apply organic amendment it in two doses, in order to provide 140 and 280 kg N ha^{-1}. Swiss chard responded positively to composts, particularly to those from animal wastes and to the higher N dose, showing a higher yield and a better product quality, while biochar did not lead to positive or negative effects. Castaldi et al. (2011) reported an increase in the exchange capacity of the soil due to the application of BC and an improvement in its ability to retain nutrients.

This study focuses on the evaluation of nitrogen rates in order to ensure high quality and environmentally friendly chard production and also to study the influence of imported carbonized plant residues as a soil amendment on the growth and development of Swiss chard.

MATERIALS AND METHODS

A pot experiment was conducted in 2018/2019 on two soil types: Vertisols and Luvisol soils. The experience is embedded in the glazed greenhouse. Plastic pots were used for the experiment. The pots was fill up with 2.5 kg of untreated or treated soil. Ten variants were developed in three replicates, as follows:

Variants	Vertisol
V1	Control- Pure soil
V2	Soil (S)+ biochar (BC)
V3	S+BC+$N_{200}P_{200}K_{250}$
V4	S+BC+$N_{400}P_{200}K_{250}$
V5	S+BC+$N_{600}P_{200}K_{250}$

Variants	Luvisol
V6	Control- Pure soil
V7	Soil + biochar (BC)
V8	S+BC+$N_{200}P_{200}K_{250}$
V9	S+BC+$N_{400}P_{200}K_{250}$
V10	S+BC+$N_{600}P_{200}K_{250}$

The tested crop is a leafy vegetable - Swiss chard (*Beta vulgaris* subsp. *vulgaris*). Chard was sown on 02.10.2018 with 20 seeds per pot.

The biochar was 6 g per pots in form of the powder mixed with the soil. The amount of biochar, phosphorus and potassium rates, are constant and serve as a background to track the impact of increasing nitrogen fertilization and the ability of the BC to absorb and release nutrients more slowly.

The introduction of mineral fertilizers was carried out with previously prepared working solutions. Norms of applied amounts of mineral fertilizers and biochar were determined based on literature data and preliminary research with chard and other leafy vegetables (Mikova et al., 2015; Stoimenov et al., 2015; Sarah et al., 2013).

The biochar used for the experiment was produced from wood chips. The pH 10.8 value of BC is strongly alkaline. It contains a large amount of carbon 61.8%, which confirms the ability of BC to deposit carbon in the soil, reducing its release into the atmosphere. The selected norms were introduced into each pot in the form of a fine powder fraction.

An optimal irrigation rate close to the field capacity was maintained through frequent watering with a small irrigation rate. The water was apply on every three day 150 ml per pots.

Phenological observations and biometric measurements were carried out during the growing season. During the chard growing season, when the rosette has reached full development, two measurements were made on 17.12.2018 and on 22.02.2019 at the end of experiment.

The nitrates, absolute dry matter in the experimental plants were determined.

Two soil types used for the experiment are Vertisols from the village of Bozhurishte (geographic coordinates: 42°45'48.59" N and 23°12'7.21" E.) and Luvisol from the village of Pozharevo (44°3′34.77 N and 26°42′23.95 E). The obtained pH data for both soil types defines them as slightly acidic. Vertisols are moderately stocked with absorbable nitrogen forms, well stocked with humus. Poorly stocked with P_2O_5 and well stocked with K_2O. The agrochemical analysis of the Luvisol from the village of Pozharevo defines it as moderately stocked with total N, slightly stocked with P_2O_5 and medium stocked with K_2O.

The following methods were used to determine the NPK in the soil:

- mineral nitrogen-extraction with 1M KCl (1:10) and distillation;
- available P and K extraction with ammonium acetate and potassium lactate with pH 4.2 (Ivanov, 1984)

Nitrates were determined from the crushed the leaves with a mortar and pestle leaf using a nitrochek meter Boeco.

RESULTS AND DISCUSSIONS

About a month after starting the experiment, the number of sprouted plants on the two soil types, according to variants, was record. The data are present in Table 1.

The largest number of sprouted plants was in variant 3 on Vertisol, and the least in variant 7 only with BC on Luvisol. In the rest of the variants, the number of sprouted plants varies from 12.26 to 14.3. The small numbers of sprouted plants in both soil types were recorded in the variants with the addition of BC only. Gomez-Eyles et al. (2013) reported that the application of biochar increased the cation exchange capacity of the soil, with NH_4 ions retaining on the BC surface (Table 1).

Table 1. Number of sprouted plants in pot experiment with Swiss chard

Variant	Number of plants per pot
Vertisols	
Var.1	13.3
Var.2	10.0
Var.3	16.7
Var.4	14.3
Var. 5	14.0
Luvisol	
Var.6	7.0
Var. 7	5.33
Var. 8	14.0
Var. 9	14.3
Var.10	13.0

The phenological development of the Swiss chard varieties was also observed. It is noticeable that growth is strongly suppressed in the different variants of Luvisol. Despite the increased nitrogen fertilization, the plants are still in the cotyledon phase, while the plants grown on Vertisol have entered the 3–4 leaf phase. In all variants, etiolating and cutting of the young plants were reported.

Variant 3 with nitrogen at 200 g/kg soil is the best on both soil types. Based on these results, we can assume that high nitrogen rates suppress the development of Swiss chard. The plants of variant 3 on Vertisol are the best developed, intensely green collared, and without lodging.

Table 2 shows the yields of fresh mass obtained in both samples. The data presented in the table correlates with the number of sprouted plants. The highest yield was reported again for var. 3 and var. 8 on both soil types, followed by var. 4. For the Luvisol, the differences in the masses of the variants fertilized with N_{400} and N_{600} are not clearly defined. The fresh mass obtained on Vertisol significantly exceeds that on Luvisol.

Table 2. Fresh mass in a pot experiment with Swiss chard

Variant	Plants mass per pot	Plants mass per pot
	I sample (g)	II sample (g)
	Vertisol	
Var.1	13.81	12.60
Var.2	13.91	8.48
Var.3	55.14	15.06
Var.4	18.31	25.51
Var.5	10.47	4 .94
	Luvisol	
Var.6	1.99	5.55
Var.7	3.54	10.31
Var.8	15.27	13.74
Var.9	4.08	6.74
Var.10	2.67	4.04

It is clear that with an increase in the nitrogen rate, the yield of fresh mass decreases, and in the second sampling, it is even lower than in the control variant. This clearly confirms the suppressive effect of higher nitrogen rates on chard development.

I crush the leaves with a mortar and pestle content of absolute dry matter (ADM) from the first sample for the different variants is in the range of 6.16% to 7.49% for the plants grown on Vertisol and 5.38% to 8.11% for those grown on Luvisol (Figure 1). In the second sampling, an increase in the ADM was observed for both soil types, with the same varying from 5.89% to 9.32% for Vertisol and from 7% to 7.96% for Luvisol.

The second reading of ADM at Vertisol shows the highest content in var. 2 with BC only, this probably due to the better water and air soil regime (Figure 1)

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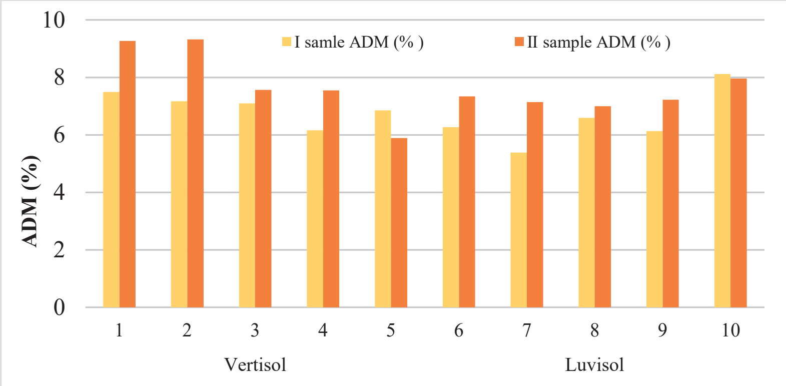

Figure 1. Absolute dry matter in chard plant samples

Mineral nutrition in plants is a process dependent on metabolism, growth, and development. During the growth processes, the supply of mineral substances to the plant increases.

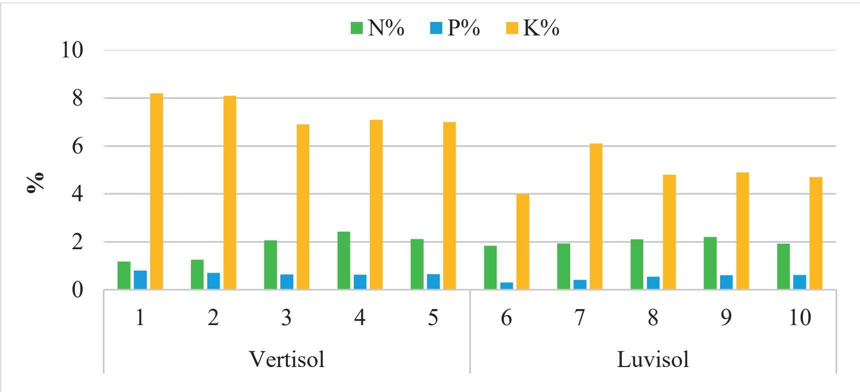

Figure 2. Percent nutrient content of Swiss chard leaves

It is clear (Figure 2) that with the increase in the nitrogen rate by variants, the nitrogen content in the leaves of Swiss chard also increases on Vertisoil. Reported values ranged from 1.18% for var. 1 to 2.4% for var. 4. The leaf N content of plants grown on Luvisol varied insignificantly between variants. This may be due to the ability of BC to equalize soil fertility and absorb nitrogen ions.

Although phosphorus is very mobile, with a constant flow from older to younger leaves, the results obtained show little variation in phosphorus content among the tested varieties. The lowest values were obtained for var. 1 control in Luvisol soils.

Of all the nutrients, potassium usually has the highest content. The large amount of potassium is associated with the carbohydrate metabolism and the water regime of the plants. In the variants grown on Vertisol, the values vary from 6.9% to 8.2%, the highest ones were recorded in var. 1 and 2, in the variants with mineral fertilization, and the values are very close. The higher rates in the first two variants are probably due to the ability of BC to change the cation exchange capacity of the soil. The other likely reason is that BC contains significant amounts of potassium in its composition. The obtained results correspond with those presented by other authors, who report that after the application of BC, the content of K in the plant biomass increased by 57%, while when manure was applied, the increase was only 43% (Lentz and Ippolito, 2012).

In the variants grown on Luvisol soil, the reported K values are smaller, varying between 4% and 6.1%, and in this soil type, the highest value was reported for var. 2 with BC only.

Based on the obtained data, it can be said that Swiss chard is a potassium-loving plant that accumulates carbohydrates in its leaf mass.

Nitrate accumulation in plants results from the uptake of nitrate ions and subsequent assimilation. Leafy vegetables do not have the nitrate-reducing ability of the roots, and this reduction takes place mainly in the leaves. Since they are used for direct consumption, Regulation (EC) No. 1881/2006 of the Commission determines the permissible amount of nitrates in the tissues to determine the concentration limit of pollutants in food. The regulation does not mention the permissible content for Swiss chard, but the specified levels for spinach and lettuce are 3500 mg NO_3/kg (Figure 3).

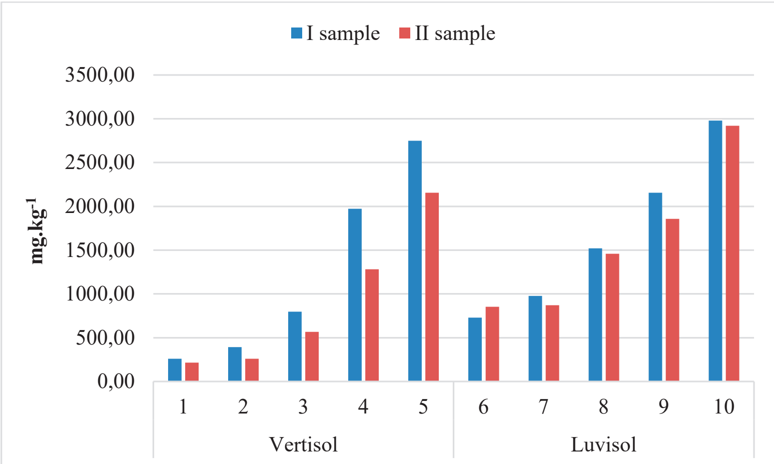

Figure 3. Nitrate content of Swiss chard leaves

In both samples on two soil types, there is a trend toward increasing nitrate content with increasing nitrogen rates. The lowest values were evaluated in the control variant, followed by the one with BC without mineral fertilization.

A gradual increase in nitrate content was reported in the Luvisol this was probably due to the acidic reaction of the soil.

Despite the detected presence of nitrates in the fresh chard leaves, the concentration does not exceed the concentration limit mentioned in Regulation 1881, which defines the product as good for consumption.

CONCLUSIONS

Based on the biometric measurements, phenological observations, and plant analyses, the following conclusions can be made:

The largest number of sprouted plants was recorded in the variant with BV+N200P200K250 on Vertisol. The fewest sprouted plants in both soil types were in the variants with biochar only and the variants with increasing rates of nitrogen fertilization.

On the Luvisol soil, plant growth is suppressed despite increasing nitrogen fertilization.

Regardless of the different starting supplies of nutrients in both soil types, the highest yield of plant mass was evaluated in the variant with BC+N200P200K250, followed by the variant with BC+N400P200K250. The fresh leaf mass recorded on Vertisol significantly exceeds that on Luvisols soil. Higher nitrogen rates have a suppressive effect on the development of Swiss chard.

As the nitrogen rate increases, so does the nitrogen content in the leaves of Swiss chard grown on Vertisol soil. The leaf N content of plants grown on Luvisol soil has a small variation. The obtained results show a slight variation in the phosphorus content in the tested variants. The lowest values were recorded for the control variant of Luvisol. The control and those with BC have the highest potassium contents. While in the versions with mineral fertilization, the values are very close. In the plants grown on Luvisol, the reported values of K were lower, with the highest content measured in the variant with only BC.

In both soil types, the nitrate content increases with the increase in the nitrogen rate, however, the concentration remains below the permissible limit.

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APPLICATIONS OF SALICYLIC ACID ON SEEDS AND SEEDLINGS OF PEPPERS UNDER SALINITY CONDITIONS

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Abstract

As a result of the global drought, improper irrigation and excessive fertilizer applications; salt accumulation in agricultural lands can cause production to stop. It is not possible to wash the soil in large lands and it is a difficult practice to rehabilitate the salty soils formed. In order to regain these soils for production, researchers have different applications; rotation and fallow practices, different irrigation methods, using of fertilizers and plant growth regulators etc. can be performed. In addition, with the changing and developing technologies, studies on production models that are not dependent on soil (soilless farming, rapid plant cultivation with LED lights in different aggregate cultures in artificially controlled environments) are being made on new models every day. This study was carried out to determine the effect of salicylic acid applications on pepper seeds and seedlings in saline conditions. Çermik pepper genotype, which is a local variety belonging to Diyarbakir, was used as plant material. Commercial rock salt was used as NaCl. While the pepper seeds were treated by keeping in 0.1mM and 0.5mM salicylic acid solution dosages, the seedlings were treated in pots containing the same doses of water culture + Hoagland nutritional solution. In order to identify the physiological and morphological changes in control and subjected to treatment plants, Chlorophyll-a, Chlorophyll-b, Total Chlorophyll content, Caretonoid content, 0-5 damage index of plants, plant fresh and dry weight (without roots), root fresh weight, plant fresh weight, leaf fresh weight, root length, stem length and stem diameter values were measured at the end of the research. When the statistical data obtained at the end of the study were examined, it was determined that both doses of Salicylic Acid increased the resistance of the plant to NaCl compared to the untreated seedlings. Furthermore, it has been determined that the application of 0.1 and 0.5mM doses of salicylic acid to the seeds and the subsequent application of SA to these seeds increases the resistance to NaCl that will be given to the environment.

***Key words**: salicylic acid, salinity, pepper, seed, seedling.*

INTRODUCTION

All kinds of extreme good or bad conditions that may occur in optimum environmental conditions in plant breeding can limit the growth and development of plants. Stress is the broad term used to describe these undesired negative situations (Gürel and Avcıoğlu, 2001).
Stress is grouped into abiotic (salinity, drought, low and high temperatures, deficiency or excess of nutrients, heavy metals, air pollution, radiation) and biotic (disease-causing fungi, bacteria, viruses and pests), which leads in low productivity and might result in losses in manufacturing output (Yağmur, 2008; Kuşvuran, 2010; Tuna and Eroğlu, 2017).
It is stated that 71% of abiotic stress and 29% of other stress factors are effective in the reduction of agricultural production due to stress (Boyer, 1982; Mahajan and Tuteja, 2005).
Salinity, one of the abiotic stress factors, negatively affects soils in agricultural production; it causes many negativities in plants grown in these soils (Bora and Deveci, 2015; Yılmaz et al., 2011; Paul and Nair, 2008). More than 800 million hectares of land in the world is affected by salinity (Munns, 2002). Approximately 1.5 million hectares of Turkey's land is faced with salinity problems (Kalefetoğlu and Ekmekçi, 2005).
This global salinity problem can cause economic losses in national incomes of countries by causing product yield and quality in crop production (Mahajan and Tuteja, 2000; Shabala and Cuin, 2008).
Considering the main cause of salinization in soils; it may result from insufficient precipitation, the presence of underground salt rocks, the intensive use of salty groundwater in agricultural production, high air temperatures and high evaporation, and unbalanced and

excessive use of salty fertilizers (Saruhan et al., 2008; Uygan et al., 2006; Ekmekçi et al., 2005). Moreover, the increase in salinity in agricultural soils, it negatively affects the water intake of the plant due to high osmotic pressure, and also disrupts the metabolism and organelles such as chlorophyll in the plant, reducing the uptake of nutrients such as K^+ , Ca^{+2} , Mn^{+2} and $NO3^-$. If any of these situations persist, the plant's growth and development will regress, its yield will drop, and it will eventually perish. (Ashraf and Bhatti, 2000; Hasegawa et al., 2000; Yıldız et al., 2010).
In an effort to lessen the negative consequences of salinity, researchers are always conducting new studies. The most important and permanent one among the searches is the selection of resistant genotypes and its recommendation to the farmers or the development of new varieties via breeding studies. Studies on the resistance of genetic resources in agricultural regions where salt issues exist are considered as the most permanent solution (Daşgan et al., 2006).
In this regard, by the researchers, especially cultivation using high-quality seeds; methods such as the use of plant growth regulators, the supplementation of various fertilizers (increasing the amount of humus in the soil by using organic fertilizers), and the application of different cultural cultivation approaches are viewed as applications to increase the plant's resistance systems under stress situations (Senaratna et al., 2000).
In agricultural lands with salinity problems, strengthening the plant's water intake, external Ca and K applications that will improve the nutrient balance, and applications that prevent the transport of Na and Cl to the upper part increase the salt resistance of the plant (Kaya and Tuna, 2005; Tuna et al., 2007; Amjad. et al., 2016; Estañ et al., 2004). The use of some healers applied externally to the plant during plant development has also been tried in recent years, and it has been reported by researchers that the applications using Salicylic Acid (SA) can have an effect on increasing the stress tolerance of the plant on various plants.
When applied externally to plants, SA plays a protective role against different abiotic stresses such as metal toxicity, high and low temperature conditions, drought and salinity, as well as curative effects on seed germination and seedling growth (Yalpani et al., 1994; Senaratna et al., 2000; Borsani et al., 2001; Rajjou et al., 2006; Alonso-Ramirez et al., 2009, Lee et al., 2010; Özdener and Kutbay, 2008; Ekinci et al., 2011). It also acts as an endogenous growth regulator of phenolic compounds (Shakirova et al., 2003). Several researches have shown that salicylic acid plays an active part in the plant's defence mechanism, pathogen resistance, flowering and flower vitality, and systemic resistance mechanism, in addition to its potential to boost plant development (Aslantaş, 2013). In a study, Salicylic acid is not required for germination under normal conditions; however, it has been reported that it promotes germination by reducing oxidative damage in high salinity environments (Lee et al., 2010).
In this study, it was aimed to determine the changes in some physiological parameters of the pepper plant in seedling period, which is moderately sensitive to salinity, by cultivating SA applied to the seeds of the pepper plant, seed + seedling and seedlings obtained from seeds that were not treated with anything. It is expected that Çermik pepper's, which is the local genotype, tolerance to salinity will be determined, and will contribute to the longevity of this genotype.

MATERIALS AND METHODS

During the spring vegetation period of 2022 the research was carried out in a plant growing cabinet belonging to Dicle University Faculty of Agriculture Department of Horticulture where the temperature, light, and humidity were all controlled.

Materials

In the study, seedlings obtained from the seeds of the Çermik pepper genotype that have been selected by us as a result of previous selection studies and are considered appropriate to work on were used as plant material.

Methods

Salicylic acid concentrations of 0.0 (control), 0.10, and 0.50 mM, as well as salt concentrations of 0.0 (control), 50, 100, and 150 mM, were utilized in the research.
Designing the trial
The following were the study subjects used in the trial:

1. Seed + control,
2. Seed + SA 0.1 mM,
3. Seed + 0.5 mM,
4. Seedling + control,
5. Seedling + SA 0.1 mM,
6. Seedling + SA 0.5 mM,
7. Seed + Seedling SA 0.1 mM,
8. Seed + Seedling SA 0.5 mM.

For salicylic acid application, pepper seeds were sown in viols containing peat: perlite (3:2) after seeds were kept in SA solutions at different doses for 12 hours. The seeds of the control group and the seeds to be applied during the seedling period were kept in a tap containing water for 12 hours; it was permissible to be planted all seeds at the same time. Two seeds were left in each cell for possible problems in seed germination; Thinning was done after germination. Seedlings consisted of SA-treated and untreated seeds were rinsed under tap water with the solids in roots 45 days after sowing and planted in 2.6 lt pots including water but no drainage. All seedlings taken into aquaculture were then supplemented with Hoagland nutrient solution, air was given to the water with aquarium pumps in order to increase the amount of oxygen in the water, and the water was completely drained once a week, and then fresh water and nutrient solution were added. Two distinct dosages of SA were administered to the pots that had been determined again at the end of the second week, and SA treatments were finished. One day following the SA application, salt was applied, and a total of 150 mM NaCl was gradually supplied to the medium over three days, 50 mM each day. Two weeks after the last salt application, the trial terminated and measurements and observations: Chlorophyll a, Chlorophyll b, Total Chlorophyll content, Caretonoid content, 0-5 damage index of plants, plant fresh and dry weight (without roots), root fresh weight, plant fresh weight, leaf fresh weight, root length, stem length and stem diameter were collected.
The experiment consisted of three replications and was carried out with 20 plants per application in each replication in accordance with the randomised plots trial design. The results were then submitted to an analysis of variance using the JUMP statistical programme.

RESULTS AND DISCUSSIONS

Salinity stress is considered to be a serious and pervasive environmental issue as well as damaging plant growth, development, yield, and other essential functions. Researchers are working to find solutions to the problems of drought and salinity, which are becoming more evident with the increasing global climate crisis, and new applications are made in aquaculture with new breeding strategies (Flowers and Yeo, 1995). One of these applications is recognized as SA. The study was terminated in the seedling period by applying 0.1 and 0.5 mM doses to the seeds of pepper plants whose regulators are sensitive to salt, which many researchers work with.
Chlorophyll-a, chlorophyll-b, total chlorophyll content, carotenoid content and damage scale in plants are presented in Table 1. The applications have been found to be statistically significant in all parameters shown in Table 1. As seen in the table, the application of SA during the seedling period caused a decrease in terms of chlorophyll a. During the seedling period, SA treatment led to a reduction in chlorophyll a content. It was discovered that the chlorophyll a content of SA applied to the seed increased in comparison to the control group. The maximum chlorophyll a content was found with SA and salt administered both to the seed and later in the seedling stage. The seed + SA 0.5 + SA 0.5 + NaCl treatment had the highest chlorophyll a content, with an increase of 66.7% over the control group. The plants in the control + salt group had the lowest chlorophyll-a concentration, with a drop of 56.86% in comparison with the control group. Similar findings were reported when the table was evaluated in terms of chlorophyll b level. With an increase of 96.72% relative to the control group, Seed + SA 0.5 + SA 0.5 + NaCl treatment had the greatest chlorophyll b concentration. In comparison to the control group, the control + salt condition had the poorest chlorophyll b level, dropping by 47.54%. When the table was examined in terms of total chlorophyll content, a similar picture emerged. While the highest value of total chlorophyll content was obtained from the seed + SA 0.5 + SA 0.5 + NaCl application with an increase of 131.6% compared to the control group plants, the lowest

value was obtained from control + salt application with a decrease of 27.4%. As a result of SA and NaCl applications, when the carotenoid content of the pepper plant seedlings was evaluated, it was discovered that this content decreased in all applications in contrast to the control group. The highest Carotenoid content was found as 1.97 in the control group; the lowest value was obtained in the control + salt application (0.76), representing a 61% drop. On the other hand, the degree of the plant damage was scored using a scale from 0 to 5. Plants with low damage severity were assessed using a 0 scale, whereas 5 scale was used for plants with high damage severity. In general, with the presence of salt given to the medium, it was determined that damage occurred in all applications compared to the control group. In the study, the best plants were determined as the plants belonging to the control group; the worst plants were obtained from control + salt application and SA + salt application given to medium afterwards, as in chlorophyll and carotenoid content. If Table 1 is summarized in general; while the doses of SA applied to the seed and the doses of SA + salt applied later in the seedling period had positive effects on the chlorophyll and carotenoid content, it was revealed that the SA + salt applications applied during the seedling period caused more damage to the plants. Tuna and Eroğlu (2017) applied SA to pepper seedlings under salty conditions in their study. Investigated the photosynthetic pigment content. Researchers stated that the total chlorophyll content of the plants in the control group was 12.51, and the chlorophyll content decreased to 5.41 as a result of NaCl application. It was stated that the total chlorophyll content was 8.72 despite the presence of salt in the medium with the presence of SA. It has also been reported that a similar situation was observed in chlorophyll a and b parameters. In the same study, in terms of carotenoid content, it was stated that while the total carotenoid content was 3.05 in the control group, it decreased to 1.81 with NaCl application. It has also been reported that the carotenoid content is 2.34, together with the presence of SA + NaCl in the medium. It is stated that the increase in salinity in photosynthetic tissues causes the breakdown of chlorophyll (Ashraf and Harris, 2004). High salinity disrupts the molecular structure of chlorophyll and reduces its amount (Deveci and Tuğrul, 2017). Bora and Deveci (2015) reported in their study to determine the physiological, morphological and chemical changes caused by different salt concentrations in pepper that the damage index of plants increased as the salinity increased.

The wet weights of leaves, stems and roots, root and stem length, and stem diameter data obtained in the study are presented in Table 2. As seen in the table, leaf fresh weights and stem fresh weights were found to be statistically significant (<0.005). While the highest values in terms of leaf fresh weight were obtained from plants belonging to the control group (1.25 g), the lowest value was obtained from plants belonging to control + salt treated pots with a decrease of 95% (0.06 g). When the table was examined, it was seen that the doses of SA applied to the seed in terms of leaf fresh weight decreased less than the doses applied to the seedling afterwards. No statistically significant difference was found in terms of root wet weights. While the highest root wet weight was detected in plants belonging to the control group (0.55 g), the lowest root fresh weight was obtained from control + salt treated plants with a decrease of 78% (0.12 g). As with the parameters of other applications, the doses of SA applied to the seed generally showed less change compared to the subsequent applications to the seedling. The highest weight in terms of stem fresh weight was obtained from plants belonging to the control group (1.67 g); It was determined that there were decreases in stem fresh weights in different doses of SA applied to plants and salt combinations. The lowest stem fresh weight was found in control + salt treatment with a decrease of 87% (0.21 g). It was recorded that treatments to be applied from seed changed less in terms of stem fresh weight than applications to be done later on seedlings. When the data on the length of the root lengths taken were examined, it was found that different applications were statistically significant on the plants (<0.001). It was observed that the longest plants in terms of root length belonged to the control group (20.32 cm). The shortest root length was obtained from plants treated with control + salt with a decrease of 75% (5.18 cm). As a result of the analyzes made in terms of

trunk length, it was seen that different applications were statistically significant (<0.001).
The longest stem length was obtained from plants belonging to the control group (15 cm); the lowest values were obtained from plants belonging to the control + salt treatment with a decrease of 60% (6.04 cm). Statistical analyzes of different applications were found to be significant (<0.005) as a result of measuring the body diameter with a digital caliper. The highest value was obtained from seed + SA 0.1 + NaCl with 1.96 mm and plants belonging to the control group with 1.92 mm.
The lowest value was obtained from control + salt application without SA application in the medium (0.90 mm). As with other parameters, it has been observed that the SA applications to be made from the seed will show less change than the SA applications to the seedling afterwards.
It has also been reported by many researchers that there are significant decreases in root fresh and dry weight, and leaf fresh and dry weight under salt stress (Tuna and Eroğlu, 2017; Erkılıç, 2005; Senaratna et al., 2000; Türkmen et al., 2002; Altunlu, 2019). Erkılıç (2005) reported that externally applied SA on pepper plants (*Capsicum annuum* L.) under salt stress had a positive effect on some physiological parameters such as fresh and dry weight of the plant. The researcher reported that there were significant drops in numerous parameters of the plants that just received salt treatment. Similar results were reported by Tuna and Eroğlu (2017) as well. To test the impact of several organic and inorganic substances on the antioxidant system in the pepper (*Capsicum annuum* L.) plant under salt stress, these two researchers treated the environment to SA.
Physiological disorders were experienced only in plants applied to salt; they stated that SA applications gave resistance to the plant. Şenay et al., (2005), in a study on wheat, reported that increasing salt concentrations reduced seedling length by 42.5% and root length by 74.4%. In their investigations on wheat, Shakirova et al. (2003) revealed that salt caused losses in the fresh and dry weights of the plants.
Yaylalı (2007) reported that the amount of salt in the irrigation water negatively affects the yield and quality characteristics of the tomato plant and the plant growth.
Aydın (2015), in his study examining the effects of salt stress on germination and seedling growth in some cultivated plants, stated that increased salt applications in all cultivars caused a significant decrease in stem and root lengths and fresh weight of the plant compared to the control.
In a study conducted to obtain the effect of SA application as an additive on salt stress, it was reported that when tomato plants are grown in SA and NaCl is added to the same medium after a certain period, salt damage to the plant can be prevented (Tari et al. 2002).

Table 1: The effects of different applications on chlorophyll, carotenoid and damage index of the plant

APPLICATIONS	Chlorophyll a	**% CHANGE**	Chlorophyll b	**% CHANGE**	T. Chlorophyll	**% CHANGE**	Carotenoid	**% CHANGE**	0-5 Scale
1. Seed + SA 0.1 + NaCl	0.67±0.00 c	**31.37**	0.96±0.00c	**57.38**	1.86±0.00 c	**95.79**	1.63±0.01 d	**-17.26**	0.40±0.31c
2. Seed + SA 0.5 + NaCl	0.75±0.00b	**47.06**	1.06±0.00b	**73.77**	2.01±0.02b	**111.58**	1.80±0.01b	**-8.63**	1.20±0.46bc
3. Seed + SA 0.1 + SA 0.1 + NaCl	0.74±0.01b	**45.10**	1.05±0.00b	**72.13**	1.98±0.01b	**108.42**	1.75±0.00 c	**-11.17**	1.00±0.29bc
4. Seed + SA 0.5 + SA 0.5 + NaCl	0.85±0.01 a	**66.67**	1.20±0.02 a	**96.72**	2.20±0.00 a	**131.58**	1.94±0.00 a	**-1.52**	0.40±0.27c
5. SA 0.1 + NaCl	0.29±0.00 f	**-43.14**	0.42±0.00 f	**-31.15**	0.85±0.00 f	**-10.53**	1.04±0.00 e	**-47.21**	1.40±0.24 bc
6. SA 0.5 + NaCl	0.36±0.00 e	**-29.41**	0.49±0.00 e	**-19.67**	1.04±0.00 d	**9.47**	0.89±0.00 f	**-54.82**	2.20±0.52b
7. CONTROL + NaCl	0.22±0.00 g	**-56.86**	0.32±0.01 g	**-47.54**	0.69±0.01 g	**-27.37**	0.76±0.01 g	**-61.42**	4.40±0.24 a
8. CONTROL	0.51±0.00 d		0.61±0.00 d		0.95±0.00 e		1.97±0.00 a		0.20±0.22c
CV	1,413889016		2.263746322		1,318101023		1,088409511		57,42914286
P	<,0001		<,0001		<,0001		<,0001		<,0001
LSD	0.0152		0.035		0.039		0.0317		1,587

Table 2: The effects of different applications on the fresh leaf, root and stem weights (g), root and stem lengths (cm) and stem diameter (mm) of the plant

APPLICATIONS	Leaf Age weight	**% CHANGE**	Root Age Weight	**% CHANGE**	Body Wet Weight	**% CHANGE**	Root Length	**% CHANGE**	Body Length	**% CHANGE**	Stem Diameter	**% CHANGE**
1. Seed + SA 0.1 + NaCl	0.80±0.22 ab	**-36.00**	0.34±0.08 a	**-38.18**	1.07±0.25 ab	**-35.93**	18.08±2.36 ab	**-11.02**	12.90±1.08 ab	**-14.00**	1.96±0.19 a	**2.08**
2. Seed + SA 0.5 + NaCl	0.55±0.10 ab	**-56,00**	0.32±0.17 a	**-41.82**	0.72±0.14 ab	**-56.89**	8.28±2.17 cd	**-59.25**	10.84±0.68 bc	**-27.73**	1.72±0.15 ab	**-10.42**
3. Seed + SA 0.1 + SA 0.1 + NaCl	0.57±0.03 ab	**-54.40**	0.16±0.02 a	**-70.91**	0.78±0.03 ab	**-53.29**	9.40±1.21 bcd	**-53.74**	11.62±0.45 abc	**-22.53**	1.66±0.09 ab	**-13.54**
4. Seed + SA 0.5 + SA 0.5 + NaCl	0.77±0.12 ab	**-38.40**	0.34±0.08 a	**-38.18**	1.25±0.26 ab	**-25.15**	16.58±1.55 abc	**-18.41**	12.34±0.81 ab	**-17.73**	1.64±0.11 abc	**-14.58**
5. SA 0.1 + NaCl	0.36±0.12b	**-71.20**	0.16±0.07 a	**-70.91**	0.50±0.15b	**-70.06**	9.68±2.17 bcd	**-52.36**	11.88±1.07 abc	**-20.80**	1.26±0.22 abc	**-34.38**
6. SA 0.5 + NaCl	0.11±0.06b	**-91.20**	0.26±0.11a	**-52.73**	0.39±0.15b	**-76.65**	7.28±1.18 d	**-64.17**	8.08±0.90 cd	**-46.13**	1.14±0.08bc	**-40.63**
7. CONTROL + NaCl	0.06±0.05b	**-95.20**	0.12±0.03 a	**-78.18**	0.21±0.05b	**-87.43**	5.18±0.62 d	**-74.51**	6.04±0.37 d	**-59.73**	0.90±0.07c	**-53.13**
8. CONTROL	1.25±0.30 a		0.55±0.19 a		1.67±0.40 a		20.32±2.60 a		15.00±0.94 a		1.92±0.23 a	
CV	64,53751901		93,02381799		61.17061663		37,29478481		17,79143179		23.86546468	
P	0.0003		0.2295		0.0016		<,0001		<,0001		0.0005	
LSD	0.726		0.519		1,001		8,973		4,065		0.763	

CONCLUSIONS

The accumulation of ions such as Na+ and Cl- caused by salinization in agricultural lands can adversely affect plant growth. Soil salinity is one of the abiotic stress factors that limit crop production. Choosing resistant cultivars is the first of the measures that must be taken when cultivating in saline soils and it is followed by implementations for strengthening salt resistance.

Salicylic acid, which ensures the continuity of the nutrient and water intake of the plants to be grown under these conditions, has been tested on many plants to increase the resistance to stress conditions. Based on our findings, salicylic acid treatments, particularly from seed or later during the seedling stage, can be suggested to reduce the effects of salt damage. In addition, the use of salicylic acid in other crop plants under salt stress can be recommended.

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STUDY ON THE EFFECT OF ACTIVATED CHARCOAL IN STIMULATING *IN VITRO* RHIZOGENESIS OF SWEET POTATO PLANTLETS

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Abstract

Activated charcoal is recommended to be added to nutrient media, used in crops of plant tissues, to the growth and development of some types of explants, but also its role of absorbing toxic compounds present in aseptic environments, which cause necrosis of inoculated tissues. . The main purpose of this study was to assess the influence of three concentrations of activated carbon (0.5 g/l, 1 g/l, 2 g/l) added to the MS culture medium, on the formation and root development of two varieties of sweet potato (DK 19/1 and DK 19/5).). A bifactorial experience was initiated, the analyzed factors being: variety and culture environment. Determinations were made on the following parameters: root length, fresh root weight and dry root weight. Based on the obtained results, an optimal rooting was observed, without callus formation, ensuring a higher percentage of capacity at acclimatization of sweet potato seedlings from in vitro to in vivo.

Key words*: sweet potato, plant tissue cultures, activated charcoal, rhizogenesis.*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam.) is a dicotyledonous vegetable belonging to the family Convolvulaceae. It is the seventh most produced crop worldwide after wheat, rice, maize, potato, barley, and cassava, and the fifth in developing countries (Jung, J.K., 2011). It is recognized as a major tuber crop (Ghasemzadeh et al., 2016) and is ranked third most important tuber after potato (*Solanum tuberosum*) and cassava (*Manihot esculentum*) (FAO, 2003). Sweet potato tubers are rich in dietary fiber, minerals, vitamins, and antioxidants such as phenolic compounds (Lebot et al., 2016; Tang et al., 2015; Teow et al., 2007). Sweet potato has recently been reevaluated as a valuable medicinal plant with anticancer, antidiabetic, and anti-inflammatory properties (Mohanraj and Sivasankar, 2014; Wang et al., 2016). The rhizoderm of the thickened roots (the tuber) has different colors: white, cream, yellow, orange, pink, red to purple, and the pulp has shades of ivory, orange, or purple-lilac (Abubakar et al., 2010; Aywa et al., 2013). Sweet potato leaves also have different shapes and colors, depending on the variety. Regarding the color of the leaves, it is usually green, yellowish-green, or with purple pigmentation. Sweetpotato leaf contains high concentrations of polyphenolics, when compared with the major commercial vegetables such as spinach, broccoli, cabbage, lettuce, and so forth. Therefore, with its tuberous roots, stems and leaves that can be fully consumed, the sweet potato is a crop that can solve food, energy, resource and environmental problems in the 21st century (Islam S., 2006). Sweet potato has received increased attention because the crop can adapt to a wide range of environmental conditions and grow on marginal areas with poor soils of limited fertility and inadequate moisture (Bioethics, 2004). For these reasons, the sweet potato crop is attractive not only to farmers but also to ecologists and economists, who are interested in developing sustainable food production systems in the tropics (Bovell-Benjamin, 2007; Loebenstein, 2009; Mukherjee et al., 2012; Iese et al., 2018). It is a new culture, which through the expansion of cultivation in our country will bring an important contribution to the diversification of vegetable products. The experimental model carried out by us in this work aims to take cuttings obtained under laboratory conditions from tuberous root shoots of some sweet potato (*Ipomoea batatas*) genotypes, with the aim of defining the optimal factors for establishing an *in vitro* culture and making some preliminary studies, regarding the development of the root system of the seedlings obtained for their good adaptability to the specific conditions of the *ex vitro* environment.

MATERIALS AND METHODS

The plant material used consists of shoots from two varieties of sweet potato provided by the Research-Development Station for Plant Culture on Sands Dăbuleni, Dolj, within the study initiated on the basis of the ADER Sectoral Project 7.3.4. The two Korean varieties of sweet potato were studied: DK 19/1 and DK 19/5. Dk/1 is a white-fleshed sweet potato genotype with a vegetation period of 120 days with the following characteristics:

- rope length - 1 m;
- total weight - 430 g;
- average number of tubers/plant - 8;
- weight of tubers/plant - 1673 g;
- tuber weight -212 g;
- tuber length - 16 cm;
- tuber diameter - 7.43 cm

Genotype DK 19/5 has tubers with butter-colored pulp with pink streaks, which turns yellow when boiled, with a vegetation period of 120 days and the following characteristics:

- rope length - 0.93 m;
- total weight - 433 g;
- average number of tubers/plant - 8;
- weight of tubers/plant - 1000 g;
- tuber weight - 125 g;
- tuber length 18 cm;
- tuber diameter - 4.86 cm;
- the plants show anthocyanin coloring on the leaves, their chlorophyll content being lower (Extract from the Technical Report/2021 - ADER Project 7.3.4).

To obtain the shoots, it was necessary to prepare the sweet potato tubers and plant them, having ensured favorable conditions of temperature, light and humidity. Watering and fertilization were carried out as necessary throughout the emergence and growth period. The pre-sprouting period was 4 weeks, after which the tuberous root buds have began to elongate, forming shoots with a length of approximately 20-25 cm. This process continued for a period of 4 months, the shoots being periodically detached from the mother plant (Figure 1) and fragmented into stem cuttings, used as a source of explants for the initiation of *in vitro* cultures (Figure 2).

Figure 1. The donor plant, source of the shoots

Figure 2. Stem cuttings

Put the cut knots of the same variety in a sterilized glass container and disinfect them with a 25% sodium hypochlorite solution and 2-3 drops of Twen 20 (R) for 15 minutes (Figure 3), operation that took place in the

hood. Then immersion in 70% alcohol, 3 minutes rinsing with double-distilled water several times, after which the explants are removed on a sterilized paper towel to remove the water. These segments were then inoculated, in test tubes containing the culture medium (5 ml/test tube) for the *in vitro* multiplication of the sweet potato (Table 1) and the amounts of activated carbon produced by Duchefa that was directly solubilized in the culture medium. In order to follow the mode of action of charcoal on the sweet potato root, a culture medium was prepared in which three concentrations of activated charcoal were added:
Culture medium: code MS (control medium);

- MS medium with an activated carbon concentration of 0.5 g/l: code MS+C1;
- MS medium with an activated carbon concentration of 1 g/l: code MS+C2;
- MS medium with an activated carbon concentration of 2 g/l: code MS+C3.

Table 1. Composition of the nutrient medium used for the inoculation of sweet potato plants

Chemical components	**Quantity /1 L mediu**
MS culture medium salts *	4.4 (g)
Ascorbic acid	0.1 (g)
Gibberellic acid	0.02 (g)
L-arginine	0.1 (g)
Calcium nitrate	0.1 (g)
Calcium pantothenate	0.002 (g)
Putrescine HCl	0.02 (g)
α-Naphthylacetic acid (NAA)	0.5 (mg)
Sucrose	30 (g)
Agar	8.5 (g)
PPM	3 (ml)

*MS – Murashige & Skoog, 1962

They were sterilized in an autoclave (20 minutes, 120^0C and pressure of 1.1-1.2 atmospheres). At the end, the samples (test tubes with explants) are obturated with tinfoil. Insert the racks with test tubes into the growth chamber, at a temperature of $25^0C\pm2^0C$, with a photoperiod of 16 hours of light and 8 hours of darkness.

Figure 3. Stem cuttings disinfection

RESULTS AND DISCUSSIONS

In the Plant Tissue Culture Laboratory, NIRDPSB Brasov, a bifactorial experiment was initiated, the analyzed factors being: the variety and the culture medium. Determinations were made on the following parameters: root length, fresh root weight and dry root weight. To obtain the results of the dry root mass, the freshly taken roots of the seedlings were kept in a room, in the dark, with a temperature of 21^0C, for 48 hours. After 10 weeks after inoculation, the obtained results were analyzed. It is recommended in the literature for its role in absorbing toxic compounds excreted by *in vitro* cultured tissues in the culture medium and causing necrosis of tissues inoculated on aseptic media. Activated charcoal is often used in plant tissue culture to improve cell division and development. It stimulates nitrogen uptake by shoots, the appearance of the nutrient medium is dark, thus promoting rooting *in vitro* (Sharma et al., 2012). Several authors have reported its beneficial effect as a culture medium supplement. This includes absorption of the phenolic complex (Pan and Staden, 1998); it is stimulatory for root formation (George and Ravishankar, 1997); rhizome development (Kim and Lee, 1992); improving seedling development (Choi and Chung, 1989); and absorption of toxic substances present in the culture medium (Fridborg and Eriksson, 1978).

In the analysis of the root length belonging to the two studied varieties, it is observed that the type of culture medium influences the growth and development process of the root. The highest average of 11.8 (cm) is found for the variety DK 19/1, on the culture medium MS+C2.

Figure 4 and Table 2 shows the results regarding the influence of the four culture medium on the growth in length of the roots of sweet potato seedlings. The best growth was recorded in the DK 19/1 variety (11.8 cm), on the MS+C2 growth medium variant.

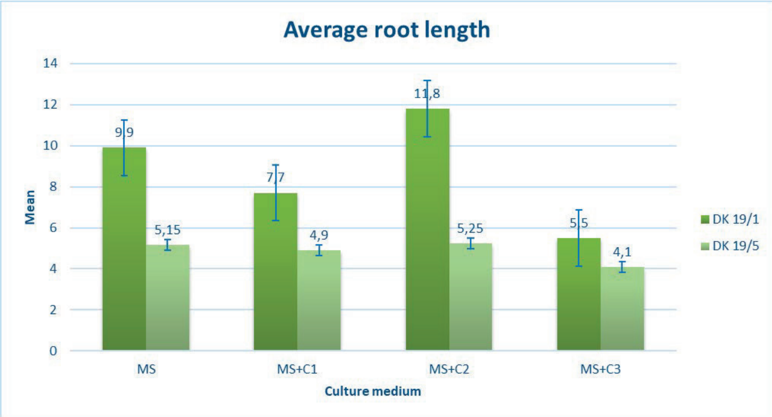

Figure 4. The influence of the culture medium with the three concentrations of activated carbon on root length

Table 2. The increase in length of the roots depending on the culture medium

Variety	Culture medium	Root length (cm)	%	Difference (cm)	Signification
DK 19/1	MS	9.90	100.0	0.00	Ct
	MS+C1	7.70	77.8	-2.20	-
	MS+C2	11.80	119.2	1.90	-
	MS+C3	5.50	55.6	-4.40	0
DK 19/5	MS	5.15	100.0	0.00	Ct
	MS+C1	4.90	95.1	-0.25	-
	MS+C2	5.25	101.9	0.10	-
	MS+C3	4.10	79.6	-1.05	-

DL 5% = 2.49 cm; 1% = 3.36 cm; 0.1% = 4.48 cm

In Figure 5, the determinations made for this studied parameter, 10 weeks after *in vitro* inoculation, show for the DK 19/1 variety, on MS+C2 culture medium, the highest average with a weight of 0.22 (g) .

In Figure 6, the determinations made for this studied parameter, 10 weeks after *in vitro* inoculation, show for the DK 19/1 variety, on the MS+C2 culture medium, the highest average with a weight of 0.22 (g).

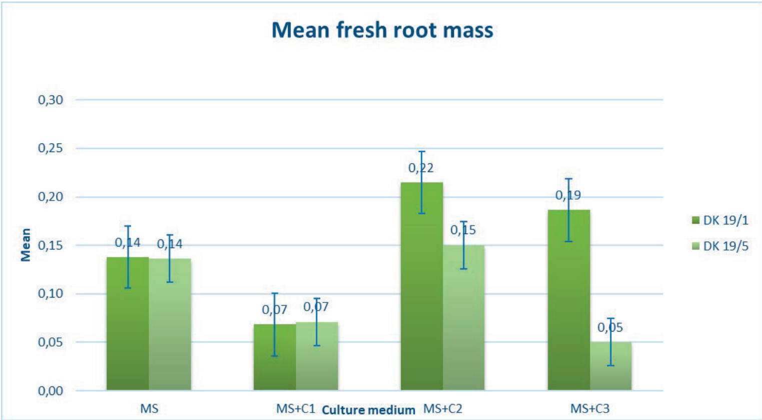

Figure 5. The influence of culture media on fresh root mass

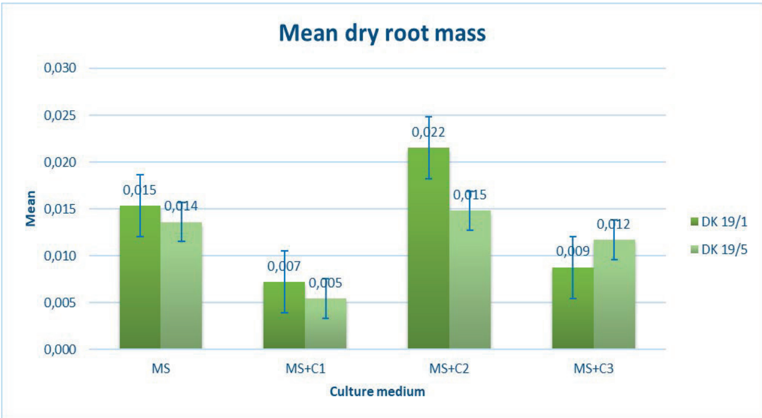

Figure 6. The influence of culture media on dry root mass

CONCLUSIONS

From the analysis of the presented results, it can be deduced that the culture environment influenced the growth and development of the roots in the two analyzed varieties. Even if the obtained values are different, they are not statistically guaranteed differences. Compared to the control sample, the best results were observed for the culture medium MSC_2 (active carbon concentration of 1 g/l), for the three parameters studied, the differentiation being factor 1, the sweet potato variety DK 19/1.

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ANALYSIS OF THE DIVERSITY OF GARLIC *(ALLIUM SATIVUM* L.) GENOTYPES FROM THE SOUTHWESTERN PART OF ROMANIA BASED ON MORPHOLOGICAL CHARACTERISTICS

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Abstract

Garlic (Allium sativum L.) is a plant species with asexual reproduction, but also with high genetic diversity. This specialized paper aims to analyse the diversity of garlic genotypes (34) identified in the southwestern part of Romania, genetic diversity based on morphological characteristics. The observations were made on morphological features, according to the standard descriptors for garlic developed by IPGRI 2001. The analysis of the variability of some morphological characteristics (7) indicated a high variability: for bulb height, the average values ranged between 1.76 cm (OT31) and 5.16 cm (CR1); for bulb diameter, the limits of variation were from 1.60 cm (CZ17) to 6.5 cm (IZ2); for the number of cloves/bulb values are between 18.8 cloves/bulb (DN34) și 6 cloves/bulb (DG11). The 34 genotypes, identified in the south area, of selected garlic, possess important morpho-quantitative and morpho-agronomic characters, some values exceeding those recorded by the control cultivar. These genotypes are valuable in terms of biological diversity, over the years adapting to environmental factors specific to the area of cultivation and will be the subject of the following studies to determine the behaviour in culture.

***Key words**: garlic, genotypes, variability.*

INTRODUCTION

Garlic (*Allium sativum* L.) is a plant species with asexual reproduction, but also with a great genetic diversity (Panthee et al., 2006). The vegetal genetic resources represent one of the most valuable resources ensuring the necessary genetic diversity for farmers and breeders. Several studies have sought after the genetic diversity of garlic, to obtain new cultivars with higher productivity, improved quality, or to acquire cultivars more adapted to abiotic stress, more resistant to pathogens and pests. To study the genetic diversity, the observations were based on morphological and physiological characters (Hirata et al., 2016; Panthee et al., 2006; Wang et al., 2014), on SSR markers (Kumar et al., 2019), RAPD (Khar et al., 2008) or AFLP markers (Ipek et al., 2006). One particular attention was paid to the identification of valuable sources of garlic germplasm with high potential for use in alternative medicine and will be the subject of the following observations to determine the behaviour in culture (Wang et al., 2014; Augusti, 1996). It is well known the role of garlic in various diseases, its antihypertensive, antidiabetic, anticancer, hypolipidemic, antimicrobial and antifungal, immunomodulatory and antioxidant, anti-inflammatory, anthelmintic, anticoagulant, and hepatoprotective potential (Londhe et al., 2011). In Romania, garlic is broadly cultivated in the household system, and over time, highly valued populations have developed a valuable base for germplasm. Many of these genotypes attract attention through their productivity, uniformity, aroma and taste, also notable for their good ecological plasticity. There are approved cultivars, but also there is a possibility to identify new valuable genotypes, starting from the valuable germplasms in rural areas. To have as much useful information about morphological and genetic characteristics used in the breeding and marketing of garlic cultivars requires precise determination and discrimination of genotypes. This paper aims to analyse the genetic diversity of some garlic genotypes identified in the southwestern part of

Romania, diversity based on morphological characteristics.

MATERIALS AND METHODS

Materials

There have been 34 genotypes of garlic (*Allium sativum* L.) analyzed, which have been identified and selected from different areas of Dolj, Olt, Mehedinți and Vâlcea counties, in 2021 (Table 1). These were coded with letters and numbers, starting with the locality from which they were selected. The selected genotypes represent old populations, which have been preserved in culture by vegetative propagation, from one year to another. For comparison, the 'Benone' variety (Vegetable Research and Development Station Buzău) was used as a control sample.

Table 1. Garlic genotypes collected from different locations (southwestern Romania)

Genotype	Locality	Coordinates	
'Benone' (control)	Buzău	45°9’N	26°49’E
CR_1, CR_{28}, CR_{29}	Cârlogani	43°31’08’’N	24°10’22’’E
IZ_2, IZ_{21}	Izvor	44°25’24’’N	23°47’57’’E
RC_3, RC_{15}, RC_{19}, RC_{20}	Răcari	44°31’27’’N	23°34’34’’E
PV_4	Piscu Vechi	43°54’00”N	23°9’56”E
SS_5, SS_{24}, SS_{32}	Șimnicu de Sus	44°23’26’’N	23°47’40’’E
GH_6	Ghindeni	44°12’41’’N	23°55’13’’E
DB_7, DB_9, DB_{18}	Dăbuleni	43°48’04’’N	24°05’31’’E
CN_8, CN_{25}	Cioroiu Nou	43°8’04’’N	23°26’2’’E
DN_{34}	Daneți	43°56’04’’N	24°03’10’’E
P_{10}	Pielești	44°19’52’’N	23°57’51’’E
DG_{11}	Drăgoaia	44°14’1’’N	23°31’32’’E
GR_{12}	Grădinari	43°33’57’’N	24°16’07’’E
OR_{13}	Orlești	44°47’26’’N	24°13’57’’E
BRT_{14}	Bratovoiești	44°7’40’’N	23°53’57’’E
CZ_{16}, CZ_{17}, CZ_{33}	Corzu	44°27’15’’N	23°10’8’’E
PS_{22}	Pișcani	44°32’31’’N	23°36’22’’E
RB_{23}	Robănești	44°18’00’’N	23°58’00’’E
GL_{26}	Gârlești	44°20’59’’N	23°54’58’’E
BL_{27}	Bălteni	44°26’51’’N	24°32’11’’E
CT_{30}	Cetate	44°07’03’’N	23°02’07’’E
OT_{31}	Caracal	44°07’N	23°21’E

Methods

The observations were made according to the standard descriptors for garlic developed by International Plant Genetic Resources Institute (IPGRI, 2001).

Regarding the bulb, the morphological characteristics studied were the following: bulb height, bulb diameter, number of cloves/bulb, bulb colour, cloves colour, bulb shape and volume.

The bulb weight was not used in the analysis, because the garlic genotypes were studied after selection from different locations, the environmental condition and duration of storage varied from one genotype to another. Therefore, the elimination of this character from the group of studied morphological characters, eliminated any possibility of statistical error in the results obtained.

The height and diameter of the bulb were measured with the digital caliper. Bulb/cloves color and bulb shape were determined according to IPGRI 2001 standard descriptors, using a color chart to indicate color intensity and the suggested method for the shape.

The obtained results were analyzed using Data Analysis, Microsoft Office Excel Programme and PCA. For each genotype were determined: the mean, the standard deviation and the coefficient of variation. The results represent an average of 50 observations/genotype.

RESULTS AND DISCUSSIONS

The obtained results regarding the variability of the morphological characters, for the 35 garlic genotypes analyzed, are presented in Table 2. From the data analysis, it is found that there is great variability from one genotype to another, useful variability in garlic breeding programs. There were significant differences in morpho-quantitative characters, in special bulb height, bulb diameter and the number of cloves in one bulb. Regarding the bulb height, the average values varied between 1.76 cm (OT31) and 5.16 cm (CR1); compared to the control sample, only the CR1 genotype's values were higher. The genotypic variability was highlighted by the coefficient of variability, the highest value belonging to the GH6 genotype (27.72%). In the control sample, the coefficient of variation had a value of 5.49%, which indicates a high uniformity for the bulb height.

Bulb diameter represents an important character in the selection activity of new genotypes.

Table 2. Summary statistics of morpho-quantitative and morpho-agronomic characteristics of the 35 genotypes of garlic*

Genotip	BH (cm)		BD (cm)		NB		V (cm^3)	
	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV
'Benone'	4.55 ±0.25	5.49	5.12 ±0.36	7.17	13.1 ±4.65	35.52	**23 ±8.23**	**35.79**
CR_1	5.16 ±0.32	6.32	5.43 ±0.73	13.60	9.83 ±1.16	11.88	28.33 ±7.52	26.56
IZ_2	4.08±0.38	9.47	6.50±0.48	7.47	14.5±2.07	14.30	43.33±5.16	11.91
RC_3	3.75±0.51	13.77	4.13±0.64	15.50	9.83±0.75	7.65	18.33±4.08	22.26
PV_4	3.35±0.27	8.17	4.06±0.3	7.56	8.83±2.31	26.22	15±5.47	36.51
SS_5	3.91±0.57	14.74	5.35±0.92	17.24	9.33±1.5	16.13	36.66±12.66	37.26
GH_6	3.60±0.99	27.72	3.31±0.37	11.34	11.16±2.13	19.12	28.33±7.52	26.54
DB_7	3.33±0.22	6.75	3.48±0.42	12.23	9.66±1.75	18.11	28.33±7.52	26.56
CN_8	3.61±0.21	5.90	3.85±0.37	9.82	10.16±1.72	16.94	41.66±4.08	9.79
DB_9	4.28±0.98	23.03	3.33±0.19	5.89	13.5±2.34	17.37	28.22±4.08	14.40
P_{10}	4.09±0.34	8.40	4.68±0.71	15.24	10.16±3.18	31.36	28.22±4.08	14.45
DG_{11}	3.35±0.21	9.25	2.40±0.12	10.0	6.00±1.65	7.5	10±0.87	8.7
GR_{12}	2.63±0.37	14.14	2.03±0.33	16.36	14.5±2.94	20.34	10±0.4	4
OR_{13}	3.28±0.64	19.49	3.53±0.34	6.85	9.16±2.78	30.40	23.33±5.16	22.13
BRT_{14}	4.16±0.92	22.23	5.0±0.39	7.89	12.0±1.50	12.90	21.66±7.52	34.74
RC_{15}	2.78±0.28	10.42	2.79±0.28	10.2	11.2±3.15	28.17	15±5.27	35.13
CZ_{16}	3.05±0.38	12.67	3.32±.28	8.72	11.4±3.13	27.49	24±5.16	21.51
CZ_{17}	1.9±0.36	19.05	1.6±0.33	20.83	12.7±3.12	24.63	10±0.35	3.5
DB_{18}	3.21±0.46	14.34	3.45±0.42	12.31	7.8±1.61	20.76	25±5.27	21.08
RC_{19}	2.83±0.35	12.58	2.26±0.30	13.55	14.2±2.29	16.19	11±3.16	28.74
RC_{20}	2.6±0.50	19.42	2.82±0.56	19.86	15±1.80	12.01	17.77±6.66	37.5
IZ_{21}	2.568±0.82	32.19	2.79±0.32	11.63	10.8±2.65	24.61	10±1.46	14.6
PS_{22}	2.46±0.22	9.23	1.86±0.15	8.28	8.3±1.33	16.11	10±0.47	4.7
RB_{23}	2.11±0.47	22.72	1.73±0.24	14.17	15.3±2.35	15.42	10±1.02	10.2
SS_{24}	2.95±0.28	9.75	2.75±0.24	8.78	11.1±2.46	22.25	17±4.83	28.41
CN_{25}	2.35±0.29	12.56	2.69±0.26	9.82	18.8±2.97	15.81	20±0.48	2.4
GL_{26}	2.04±0.21	10.38	2.52±0.31	12.52	8.9±3.14	35.31	11±3.16	28.74
BL_{27}	2.48±0.22	9.07	2.85±0.25	8.94	12.2±2.89	23.75	20±3.05	15.25
CR_{28}	2.87±0.46	16.29	3.32±0.33	10.07	9.42±3.40	36.15	24.28±5.34	22
CR_{29}	2.59±0.61	23.83	2.94±0.33	11.36	13.5±3.24	24	21±3.16	15.05
CT_{30}	2.23±0.23	10.34	2.23±0.05	2.58	13.33±2.30	17.32	10±1.64	16.4
OT_{31}	1.76±0.20	11.78	2±0.2	10	12±1	8.33	10±0.86	8.6
SS_{32}	3±0.70	23.57	3.05±0.47	15.48	13±2.28	17.54	16.66±8.16	48.98
CZ_{33}	1.91±4.88	25.58	2.5±0.60	24.07	11.5±2.79	24.33	13±4.83	31.15
DN_{34}	2.88±0.27	8.56	2.86±0.22	6.43	18.8±2.09	27.96	20±2.75	13.75

*Note: BH = bulb height (cm), BD = bulb diameter (cm), NC = the number of cloves/bulb, V = volume (cm3); SD = Standard Deviation; CV = coefficient of variation.

The analysis of the obtained data shows that the limits of variation for this characteristic were between 1.60 cm (CZ_{17}) and 6.5 cm (IZ_2). Compared to the control cultivar, in 3 of the 34 genotypes analyzed the values were higher (IZ_2-6.50 cm, CR_1-5.43 cm, SS_5-5.35 cm). The coefficient of variation varied between 5.89% (DB_9) and 24.07% (CZ_{33}), which indicates a small and medium variability for this character. The medium values obtained for this character to the IZ_2 (6.5 cm), CR_1 (5.43 cm), SS_5 (5.35 cm) and BRT_{14} (5 cm) genotypes, were higher than the one reported by Singh (2014) for a garlic genotype (LS01). The uniformity of this character represents the practical importance of the marketing of garlic bulbs.

Regarding the number of cloves/bulb was influenced by genotype, ranging from 18.8 cloves/bulb (DN_{34}) and 6 cloves/bulb (DG_{11}), while the control cultivar records an average of 13.1 cloves/bulb. The coefficient of variation indicated low-high variability within genotypes, ranging from 7.5% (DG_{11}) to 36.15% (CR_{28}). A large variability for this characteristic (4-55 cloves/bulb) was highlighted by using the observation made by Panthee et al. (2006) of garlic genotypes in Nepal.

The present results, regarding bulb equatorial diameter and the number of cloves/bulb are in agreement with the results of the previous researchers, which reported a broad range of variations of the morphological characters of the bulb to the garlic genotypes studied (Khar et al., 2006; Vatsyayan et al., 2013; Wang et al., 2014; Ranjitha et al., 2018; Tesfaye, 2021). The volume of the bulb was different from one genotype to another, from a minimum of 10 cm^3 (DG_{11}) to a maximum of 43.33 cm^3 (IZ_2) compared to the control cultivar which has an average value of 23 cm^3. In reference to the colour of the bulb (Table 3), if we talk about the 35 genotypes, including the control sample, the dominant colours were: white (7 genotypes: CR_1, PV_4, BRT_{14}, SS_{24}, BL_{27}, OT_{31}, DN_{34}), cream (19 genotypes: RC_3, SS_5, GH_6, DB_7, CN_8, P_{10}, DG_{11}, OR_{13}, RC_{15}, CZ_{16} RC_{19}, RC_{20}, IZ_{21}, PS_{22}, RB_{23}, CR_{28}, CR_{29}, CT_{30}, CZ_{33}), beige (6 genotypes: Benone, CZ_{17}, DB_{18}, CN_{25}, GL_{26}, SS_{32}), light violet (1 genotype: IZ2), a heterogeneous population with differently coloured bulbs (2 genotypes: DB9, GR12). It is found that of the 15 genotypes studied, 8 had cream-coloured parchment leaves. This colour dominates over 50% of the genotypes studied, an observation similar to that of Stavělíková (2008) in a study of a collection of 613 garlic genotypes. If we consider the colour of the cloves (Table 3), yellow/light brown (22 genotypes: 'Benone', CR_1, RC_3, SS_5, DB_7, CN_8, P_{10}, BRT_{14}, RC_{15}, DB_{18}, RC_{19}, RC_{20}, IZ_{21}, PS_{22}, RB_{23}, SS_{24}, CN_{25}, GL_{26}, BL_{27}, OT_{31}, DN_{34}, SS_{32}, CZ_{33}), brown (2 genotypes: GR_{12}, CZ_{17}), violet (8 genotypes: IZ_2, GH_6, DB_9, DG_{11}, CR_{28}, CR_{29}, CT_{30}, CZ_{16}), a heterogeneous population with differently coloured cloves (2 genotypes: PV_4, OR_{13}). Fossen & Andersen (1996) consider that the violet colour is due to the accumulation of anthocyanins. Also, the shape of the bulb varied within the genotypes analysed (Table 3). The genotypes (23) that had a circular shape are: CR_1, PV_4, GH_6, DB_7, DB_9, GR_{12}, RC_{15}, CZ_{17}, DB_{18}, RC_{19}, RC_{20}, IZ_{21}, PS_{22}, RB_{23}, CN_{25}, GL_{26}, BL_{27}, CR_{28}, CR_{29}, CT_{30}, OT_{31}, DN_{34}, SS_{32}. The broadly ovate shape is found in: Benone, IZ_2, RC_3, CN_8, P_{10}, DG_{11}, OR_{13}, BRT_{14}, SS_{24}, CZ_{16}, CZ_{33}. Only one genotype has a heart-shaped bulb (SS_5).

The correlation analysis helps to evaluate the relationship between the morphological characteristics of the bulb for the studied genotypes. The correlation coefficients between the morphological characteristics of garlic genotypes (bulb height, bulb diameter, the number of cloves/bulb, volume) are shown in Table 4. The bulb diameter was moderate correlated with the height of the bulb (r=0.852). A negative correlation between the number of cloves/bulb and the bulb diameter (r=-0.157) indicates that genotypes with large bulb weight may not necessarily produce more cloves/bulb. The results obtained in this study, regarding the correlations between the morphological characteristics of the selected garlic genotypes, according to the literature (Panthee et al., 2006; Wang et al., 2014). Given that a variable is feasible for the direct selection of garlic cultivars, it must have a direct effect on production yield and a high correlation leading in the same direction to the yield of garlic bulbs. In this sense, the variables of the equatorial diameter of the bulb and its height are the most suitable for the direct selection of the most productive garlic genotypes, because they have a cause-effect relationship with the crop yield.

Table 3. The shape and colour of bulbs in the analysed garlic genotypes

No.	Genotype	Bulb colour/Code-Colour	Cloves colour/Code-Colour	Bulb shape/Code-Shape
1.	'Benone'	3-beige	2-yellow/light brown	3-broadly-ovate
2.	CR_1	1-white	2-yellow/light brown	1-circular
3.	IZ_2	5-violet deschis	5-violet	3-broadly-ovate
4.	RC_3	2-cream	2-yellow/light brown	3-broadly-ovate
5.	PV_4	1-white	99	1-circular
6.	SS_5	2-cream	2-yellow/light brown	2-heart shaped
7.	GH_6	2-cream	5-violet	1-circular
8.	DB_7	2-cream	2-yellow/light brown	1-circular
9.	CN_8	2-cream	2-yellow/light brown	3-broadly-ovate
10.	DB_9	99	5-violet	1-circular
11.	P_{10}	2-cream	2-yellow/light brown	3-broadly-ovate
12.	DG_{11}	2-cream	5-violet	3-broadly-ovate
13.	GR_{12}	99	3-brown	1-circular
14.	OR_{13}	2-cream	99	3-broadly-ovate
15.	BRT_{14}	1-white	2-yellow/light brown	3-broadly-ovate
16.	RC_{15}	2-cream	2-yellow/light brown	1-circular
17.	CZ_{16}	2-cream	5-violet	3-broadly-ovate
18.	CZ_{17}	3-beige	3-brown	1-circular
19.	DB_{18}	3-beige	1-white	1-circular
20.	RC_{19}	2-cream	2-yellow/light brown	1-circular
21.	RC_{20}	2-cream	2-yellow/light brown	1-circular
22.	IZ_{21}	2-cream	2-yellow/light brown	1-circular
23.	PS_{22}	2-cream	2-yellow/light brown	1-circular
24.	RB_{23}	2-cream	2-yellow/light brown	1-circular
25.	SS_{24}	1-white	2-yellow/light brown	3-broadly-ovate
26.	CN_{25}	3-beige	2-yellow/light brown	1-circular
27.	GL_{26}	3-beige	2-yellow/light brown	1-circular
28.	BL_{27}	1-white	2-yellow/light brown	1-circular
29.	CR_{28}	2-cream	5-violet	1-circular
30.	CR_{29}	2-cream	5-violet	1-circular
31.	CT_{30}	2-cream	5-violet	1-circular
32.	OT_{31}	1-white	2-yellow/light brown	1-circular
33.	SS_{32}	3-beige	2-yellow/light brown	1-circular
34.	CZ_{33}	2-cream	2-yellow/light brown	3-broadly-ovate
35.	DN_{34}	1-white	2-yellow/light brown	1-circular

Table 4. Correlation matrix - Pearson (n) between the morphological characteristics of garlic genotypes*

Variables**	BH	BD	NC	V
BH	**1**	0.852	-0.225	0.686
BD	0.852	**1**	-0.157	0.786
NC	-0.225	-0.157	**1**	-0.062
V	0.686	0.786	-0.062	**1**

*Values in bold are different from 0 with a significance level alpha = 0.05; **BH = bulb height (cm), BD = bulb diameter (cm), NC = the number of cloves/bulb, V = volume (cm3).

In order to reduce the dimensionality of the data set, they were subjected to Principal Component Analysis, a method that revealed the most predominant variables. To study the diversity, the method was used, also, by authors, to garlic, but also other species (Jabbes et al., 2012; Cosmulescu and Botu, 2012). Table 5 presents the statistical processing of the data obtained regarding the main characteristics analyzed. There is a high and very high validity for the analysed characteristics, the variability highlighted by the coefficient of variation, whose values exceed 19.36%.

Table 5. Summary statistics for the 6 characteristics analysed

Variable	Min	Max	Mean	Std. dev.	CV%
Bulb height (cm)	1.7	5.1	3.07	0.81	26.58
Bulb diameter (cm)	1.6	6.5	3.27	1.16	35.44
The number of cloves/bulb	6.0	18.8	11.74	2.82	24.05
Volume (cm3)	10.0	43.3	20.0	9.08	45.41

PCA analysis for the 4 morphological characteristics analysed (bulb height, bulb diameter, the number of cloves/bulb, volume) showed that the first 3 characteristics (F1- bulb height, F2-bulb diameter, F3- the number of cloves/bulb) represented 96.83% of the total variability (Table 6).

Table 6. Eigenvalues and component score coefficients

	F1	F2	F3	F4
Eigenvalue	2.59	0.97	0.30	0.12
Variability (%)	64.83	24.42	7.58	3.16
Cumulative (%)	64.83	89.25	96.83	100.00

The chart accomplished based on the values obtained on the first 2 axes (F1- bulb height. F2- bulb diameter; Figure 1) suggests the existence of 4 groups. and the presented variability represents 89,26% of the total variability.

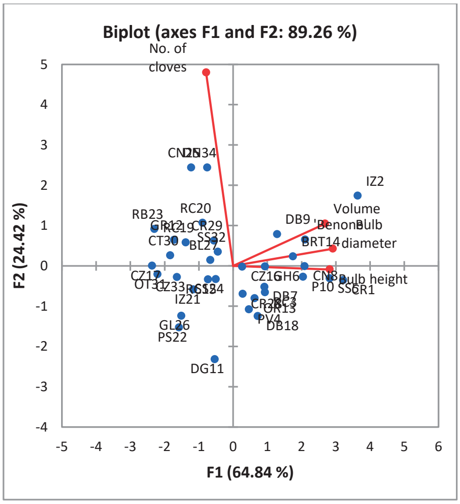

Figure 1. Two-dimensional analysis of the main components representing the F1 and F2 axes illustrating the variation of the characteristics of the bulb height (cm) and bulb diameter (cm) in 35 garlic genotypes

Group I, which consists of 9 genotypes, with negative values for both components, includes the following genotypes: CZ_{17}, DG_{11}, SS_{24}, RC_{15}, IZ_{21}, CZ_{33}, GL_{26}, PS_{22}, OT_{31}. Group II consists of 12 genotypes grouped in positive values for F1 and negative values for F2 (CZ_{16}, CR_{1}, GH_{6}, SS_{5}, OR_{13}, CR_{28}, DB_{7}, RC_{3}, P_{10}, PV_{4}, DB_{18}, CN_{8}). Group III includes 3 genotypes grouped in positive values for both components (BRT_{14}, DB_{9}, IZ_{2},) and the control variety 'Benone'. Group IV includes 10 genotypes grouped in positive values for F2 and negative for F1 (CN_{25}, DN_{34}, GR_{12}, RC_{20}, RB_{23}, RC_{19}, CR_{29}, CT_{30}, BL_{27}, SS_{32},) (Figure 1).

In the figure based on the obtained values at the axes F2 (bulb diameter) and F3 (number of cloves/bulb), are shown 4 groups of genotypes; the present variability represents 32.00% of the total variability (Figure 2).

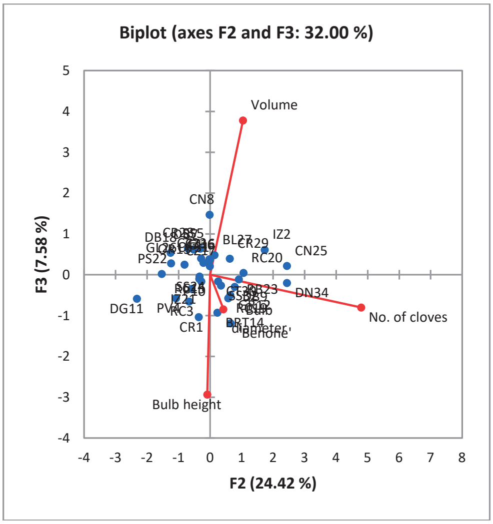

Figure 2. Two-dimensional analysis of the main components representing the F2 and F3 axes illustrating the variation of the characteristics of the bulb diameter (cm) and number of cloves/bulb in 35 garlic genotypes

Group I, consists of 7 genotypes, with negative values for both components, including the genotypes DG_{11}, IZ_{21}, RC_{15}, P_{10}, PV_{4}, RC_{3}, SS_{24}, CR_{1}. Group II is composed of 8 genotypes grouped in positive values for F2 and negative values for F3 (BRT_{14}, RC_{19}, SS_{32}, CT_{30}, GR_{12}, DB_{9}, DN_{34}, RB_{23}) and the control cultivar 'Benone'. Group III consists of 5 genotypes grouped in positive values for both components (BL_{27}, IZ_{2}, CN_{25}, RC_{20}, CR_{29}). Group IV consists of 14 genotypes grouped in positive values for F3 and negative values for F2 (CR_{28}, OR_{13}, GL_{26}, PS_{22}, DB_{7}, CZ_{17}, SS_{5}, CN_{8}, DB_{18}, CZ_{33}, GH_{6}, CN_{8}, GH_{16}, OT_{31}) (Figure 2).

The original features, which have nothing to do with the main components, are perpendicular (or almost perpendicular) to them, or are reflected in short lines ending near the origin. Therefore, the feature number of bulbs is least associated with the height of the bulb; the bulb volume feature is little associated with the bulb diameter (although it demonstrates some positive correlation with this component) (Figure 1). The feature of the number of cloves/bulb is the least associated with the height and diameter of the bulb (Figure 2).

CONCLUSIONS

In conclusion, the study shows the existence of a large variability of morphological characteristics present in some selected garlic genotypes from southwestern Romania. The 34 selected genotypes of garlic have important morpho-quantitative and morpho-agronomic characteristics, some values exceeding those recorded by the 'Benone' cultivar, taken as a control. Those genotypes are valuable from the point of view of biological diversity, over the years adapting to the environmental factors specific to the area of origin and will be the subject of the following observation to determine the behaviour in culture.

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EFFECT OF BIOFERTILIZERS ON QUALITY OF SWEET CORN

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Abstract

Paper presents the effect of the Panoramix biofertilizer, whose composition consists of a combination of fungi (Trichoderma spp.) and bacteria (Bacillus spp.) on the quality of the edible part of sweet corn. The sweet corn crop was established in 2019, by sowing, and the seeds were treated with Panoramix, at a dose of 2 ml/kg seed. In this sense, two sweet corn hybrids were tested:`Basin $F_1$` and `Challenger $F_1$. The variants were organized as follows:`V_1- `Basin $F_1$`- untreated; V_2- `Basin $F_1$`- treated; V_3- `Challenger $F_1$`- untreated; V_4 -`Challenger $F_1$` - treated. The results regarding some the quality components, like TSS, reducing sugar, starch, carotene and antioxidant capacity confirmed the superiority of the application of the Panoramix biofertilizer compared to non-mycorrhizal variants. Among the tested hybrids, `Challenger $F_1$` recorded a better quality of sweet corn caryopses in the version treated with the micorryzal product. Based on the obtained results, it can be stated that the product Panoramix could be recommended as a promising sustainable approach to stimulate the quality of the sweet corn. Among the tested hybrids, `Challenger $F_1$` recorded a better quality of sweet corn caryopses in the version treated with the with the mycorrhizal product.

***Key words**: Zea mays* var.*saccharata, mycorrhiza, production quality.*

INTRODUCTION

Sweet corn (*Zea mays* var. *saccharata*) is a vegetable grown mainly for human consumption, eaten fresh or processed. It is a rich an important source of fiber, minerals, vitamins, folic acid, amino acids, proteins, antioxidant compounds (polyphenols and carotenoids) and other phytochemicals with bioactive properties. There are numerous studies that confirm the presence of phenolic and flavonoid compounds in sweet corn. Among the predominant phenolic acids are ferulic and p-coumaric acid, but caffeic, syringic, vanillic and gallic acids have also been identified. Among flavonoids, the major compounds are kaempferol and quercetin (Das and Singh, 2016).

Sweet corn is a valuable source of carotenoids that have the property of protecting cells against oxidative stress (O'Hare et al., 2015). Beta carotene has an important role in supporting the immune system, in the process of cell specialization and metabolism of iron (Young and Woodside, 2001). Lutein and zeaxanthin are the major carotenoids found in sweet corn caryopses (Pacurar-Grecu et al., 2017; O'Hare et al., 2015; Junpatiw et al., 2013), known for their importance in maintaining eye health to protect ocular tissues from phototoxic damage (Bone et al., 2000).

In recent years, more and more intensive practices have been applied in agriculture that increase crop yields, practices with a negative impact on the environment. For increasing agricultural yield it must use rationally doses of fertilizer (Iancu et al., 2019). Therefore, sustainable and ecological technologies should be applied more often in agriculture to reduce the negative effects on the environment. From this point of view, biostimulators are a viable alternative to achieve this purpose. These products have many mechanisms of action, such as: stimulating seed germination, developing the root system, increasing resistance to diseases and pests, mitigating biotic and abiotic stress, increasing nutrient solubility or influencing photosynthetic activity. In the category of biostimulators are also included products based on mycorrhizas that contain fungi of the genus *Trichoderma* and bacteria of the genus *Bacillus* spp.

In addition, arbuscular mycorrhizal fungi (AMF) also function as a bioregulator with a role in the phytohormonal balance influencing various production quality parameters (Antunes, 2012). The most pronounced effects are observed in horticultural products with a high content of phytocompounds with beneficial effects on health (carotenoids, flavonoids, polyphenols), research being focused on the selection of AMF species that intensify secondary metabolism and lead to increased nutritional value. Rouphael et al., (2015), carry out a synthesis study in which they present the improving effects of arbuscular mycorrhiza on the growth and productivity of some horticultural crops.

Other microorganisms used as biostimulators are *Trichoderma* spp. fungi, being considered as the most important filamentous fungus in the biocontrol strategies of plant growth inducing resistance to the incidence of diseases. It controls a broad group of phytopathogens through mycoparasitism, using specialized attachment structures, enzymatic lysis and synthesis of secondary metabolites to compete for resources (Geraldine et al., 2013; Kohl et al., 2019). Different species of *Trichoderma* show a good adaptability, efficient reproductive capacity and the ability to survive in unfavorable growing conditions, but also rapid use of available nutrients from the soil. It is an active component in many biopesticides, biofertilizers and biostimulants, with beneficial effects on many horticultural crops: tomatoes (Molla, 2012) brassica plants (Poveda et al., 2019), lettuce (Avio et al., 2017), bean (Manzar et al., 2022), corn (Nascimento et al., 2020), etc.

Among the microorganisms with biostimulating properties of special interest is the bacteria which associated with the roots and the rhizosphere produce plant hormones (auxin, gibberellin and cytokinin), organic compounds and enzymes, antimicrobial compounds, fix nitrogen, increase the absorption of water and nutrients, induce systemic resistance, suppresses pathogens and ensures plant growth. These are called plant-growth-promoting bacteria-PGPB (Ruzzi and Aroca, 2015). Among these diverse microorganisms, *Bacillus* spp. have become some of the most attractive and effective agents of bacterial control for plant growth stimulation.

The interaction of plants with beneficial microorganisms can lead to a significant accumulation of secondary metabolites in the edible parts of plants, which are nutritionally valuable functional compounds, such as: ascorbic acid, phenolic compounds, terpenoids, carotenoids (Ganugi et al., 2021). Stimulation of secondary metabolism when applying biostimulators is also reported by other authors. In the study by Katsenios et al. (2022) that investigated the effectiveness of 10 strains of plant growth promoting bacteria on sweet corn crop, they observed that treatment with *Bacillus licheniformis* caused an increase in photosynthetic rate, transpiration rate and stomatal conductance during the cultivation period. Also, by using the Panoramix product (a mixture of *Bacillus* spp. and *Trichoderma* spp.) in the wheat culture, seed germination and the physiological processes involved in plant growth were improved (Ayed et al., 2022).

There are numerous claims regarding the effect on plant metabolism through the use of biofertilizer products that synergistically combine the beneficial effects of different species of *Trichoderma* and *Bacillus* spp. (Molla et al., 2012; Efthimiadou et al., 2020). The use of beneficial microorganisms in agriculture, often referred to as microbial biostimulants, is gaining popularity in recent years as a sustainable approach to promote plant growth and productivity (Ganugi et al., 2021). El Fattah et al. (2023) report the positive effects of arbuscular mycorrhiza on the morphological and production characteristics of the sweet corn crop. In contrast, there is limited information in the literature regarding the effect of AMF on biochemical compounds in sweet corn. As a result, the research in this paper followed the effect of the application of Panoramix biostimulator on the nutritional quality of caryopses in two sweet corn hybrids.

MATERIALS AND METHODS

The biological material was represented by sweet corn hybrids: Basin F_1 and Challenger F_1. In this regard, the seed was treated with

Panoramix, a biological complex whose composition consists of a combination of fungi (*Trichoderma* spp.) and bacteria (*Bacillus* spp.) that promote plant growth and their resistance to various environmental factors. These microorganisms colonize the roots and protect the crop during the entire vegetation period ensuring the availability of nutrients more easily, such as nitrogen and phosphorus. The experimental model included four variants: V_1-`Basin $F_1$` untreated; V_2- `Basin $F_1$` treated; V_3-`Challenger $F_1$` untreated and V_4-`Challenger $F_1$` treated. The sweet corn crop was established in the third decade of April, in 2019, applying the technological sequences specific to this crop.

The biochemical determinations were carried out at technical maturity (ready for consumption), in the milk-wax phase of the caryopses and focused on the content of: SUS, reducing sugar, starch, vitamin C, carotene, total phenolic compounds and antioxidant activity.

Chemical analysis

Total soluble solids content (TSS) (%) was determined using a digital refractometer (Kruss Optronic DR 301-95) at 20°C.

The reducing sugars were extracted in distilled water (1:50 W/V), 60 minutes at 60˚C and determined by the colorimetric method at 540 nm with 3,5-dinitrosalicylic acid reagent using glucose as standard (Soare et al., 2019). The results were expressed in % fresh weight basis.

The starch content was determined by using Ewers polarimetric method (Soare et al., 2019). Starch from the ethanol-insoluble material is extracted into hot dilute hydrochloric acid. After having cooled, phosphotungstic acid is added to precipitate the proteins and the solution is filtered. The optical rotation of the filtrate is measured using a Carl Zeiss JENA polarimeter and the results were calculated with a specific optical rotation of the starch [α] D20˚ = 184.6˚. The results were expressed in % fresh weight (FW) basis.

Total carotenoid content was determined with the spectrophotometric method (Babeanu et al., 2016). For extraction of carotenoids 1 g fresh material was homogenized with 10 mL acetone. The acetone extract was mixed with 10 mL hexane, and then 25 mL water was added to separate carotene into the hexane layer. Absorbance at 450 nm was measured and total carotenoid content is calculate using a value of 2500 for the extinction coefficient (E1%) and expressed as mg/100 g FW.

Ascorbic acid was extracted in 2% hydrochloric acid, HCl; 5:50 w/v (Soare et al., 2018). The determination of ascorbic acid is performed from the supernatant with iodometric redox titration in which iodine reacts with ascorbic acid, oxidizing it to dehydroascorbic acid. The ascorbic acid content was expressed as mg/100 g FW.

The extracts for the determination of total phenolic content and antioxidant activity were prepared into 80% aqueous methanol (1: 10 w/v) at 24°C for 16 h.

Total phenolic compounds (TPC) content was determined colorimetric at 765 nm by using the Folin-Ciocalteu method (Babeanu et al., 2016) based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalents (GAE)/100 g FW.

Antioxidant activity is evaluated as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay by colorimetric method (Soare et al., 2018). 2 mL of 0.075 mM DPPH methanolic solution was mixed with 0.1 mL extract and vortexed thoroughly. The absorbance of the mixtures was measured at 517 nm after 20 minutes. A blank reagent was used to study stability of DPPH over the test time. The percentage of DPPH radical scavenging activity of extracts was evaluated according to the formula: % scavenging = $[A_0-(A_1–A_S)]/ A_0]100$, where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only. Trolox (T) was used as standards. The standard calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The results were expressed as mmol Trolox equivalents (TE) per 100 g fresh weight (mmol TE/100 g FW).

The spectrophotometric measurements were performed with Evolution 600 UV - Vis spectrophotometer, Thermo Scientific, England with VISION PRO software. All determinations were performed in triplicate, and all results were calculated as mean.

The processing of the results was carried out according to the method of analysis of variance. The comparisons of means were calculated using the method of multiple comparisons, at a level of significance $P \leq 0.05$. Mean values on the same column indicated by different letters are statistically different.

RESULTS AND DISCUSSIONS

The result regarding the effect of the Panoramix biostimulator on the quality characteristics (TSS, reducing sugar, carotene, ascorbic acid, total phenols and antioxidant activity) of sweet corn caryopses are presented in Tables 1 and 2. Thus, TSS varied from 12.72% (V_2-`Basin $F_1$`) to 16.85% (V_3-`Challenger $F_1$`). From the recorded results, it can be seen that the reaction of the hybrids to the applied biostimulator is diverse. Application of the product caused an increase in TSS content only in `Challenger $F_1$`. Also, Efthimiadou et al. (2020) reported that through soil and foliar application of the PGPB biostimulator there was an increase in the content of soluble substances in corn seeds and crude fiber in the treated variants, and Nascimento et al. (2020) observed a significant increase in the amount of total soluble and dry substance on corn (*Zea mays*) in the biofertilized variants compared to the control.

The main attribute that makes sweet corn attractive to the consumer is the sweet taste determined by the sugar content. Sweet corn contains glucose, fructose and mostly sucrose. Soluble sugars in sweet corn contain 94.88 and 85% sucrose in genotypes *sh2*, *se* and *su* (Becerra Sanchez and Taylor, 2021), and glucose and fructose in smaller quatities.

In the present study, the reducing sugar in the corn caryopses varied from 1.89% (`Basin $F_1$`) to 3.16 % (`Challenger $F_1$`) (Table 1.). Comparing the hybrids between them, it is found that `Challenger $F_1$` recorded high values compared to `Basin $F_1$` for both variants, and regarding the influence of the treatment, there was an increase in the level of reducing sugar in both hybrids, in the treated variants. Thus, it can be said that sugar accumulation can be influenced by the hybrid, technology, environmental conditions and harvest time.

Our results are also supported by Bona et al., (2018) who showed the positive effect of applying AMP to the tomato crop, by increasing the concentration of sugar in the fruit, and by Molla et al. (2012) through biofertilization with BioF/compost (household/kitchen wastes composted by *Trichoderma harzianum* T22).

Table 1. The content of some quality indices in sweet corn

The specifics of the variants	TSS (%)	Reducing sugars (%)	Starch (%)
V_1- `Basin $F_1$`- untreated	13.21^c	1.89^b	14.28^{ns}
V_2- `Basin $F_1$`- treated	12.72^d	1.94^b	16.45 ^{ns}
V_3-`Challenger $F^1$` - untreated	16.85^a	2.98^a	15.06 ^{ns}
V_4- `Challenger $F_1$` -treated	16.24^b	3.16^a	13.66 ^{ns}
LSD ($P \leq 0.05$)	0.34	0.71	6.48

The starch content varied between 13.66% in (V_4) and 16.45% in (V_2) (Table 1). In the case of the `Basin $F_1$` hybrid, the starch content was higher in the treated variant compared to the untreated variant, while in the `Challenger $F_1$` hybrid, lower values were recorded in the case of the treated variant. From a statistical point of view, there are no significant differences between the variants. Some authors reported a variable content of starch in potatoes, influenced by the variety and the type of biofertilizer (Mystkowska, 2019). Also, Katsenios et al. (2022) who investigated the effect of some strains of PGPB (plant growth promoting bacteria), on the quality of sweet corn, reported that only certain strains such as *B. mojavensis*, *B. subtilis* and *B. pumilus* influenced grain quality (protein and fiber content), and other strains positively influenced production. These researches are useful to highlight the different influence of mycorrhizal fungi depending on the strain.

The content of ascorbic acid in sweet corn varied between 6.48 mg/100 g FW in (V_1) and 7.79 mg/100 g FW in (V_4). Comparing the two hybrids, significant differences are found between the treated and the untreated variants or between the same variants. In both hybrids, the treatment with biostimulator led to an increase in the ascorbic acid content. Similar observations were also reported by Apostol et al., (2022) who found a significant increase in the culture of long pepper fertilized with mycorrhizal fungi compared to the unfertilized version or the one fertilized with fulvic acids. José David et al., (2018) showed that inoculation with strains of *Phyllobacterium* and *Rhizobium* is a good agronomic practice, which improves the content of vitamin C and other bioactive compounds in strawberries, with beneficial effects on human health.

Secondary metabolites of plants are considered key bioactive compounds for a healthy diet (Avio et al., 2017).

Regarding the content of total carotene in sweet corn, the higher values were recorded in the treated variants 0.89 mg/100 g FW at V_2 and 0.98 mg/100 g FW at V_4 respectively. In both hybrids, the treatment does not induce a significant increase in the total content of carotenoids. The effect of biostimulators with AM and PGPB fungi used in combination was also studied on the nutritional quality of tomatoes, indicating an increase in the concentration of carotene in fruits (Bona et al., 2018). Some studies have shown the contradictory effect on the concentration of carotene under the influence of mycorrhizal inoculation. Horváth et al. (2020) in their study they evaluated of mycorrhizal product containing AM mixture on tomato culture, in conditions of insufficient irrigation and showed a decrease in total carotene content. It can be appreciated that the variability of the results is due to the influence of different inoculated mycorrhizal fungi.

Table 2. Antioxidant activity in sweet corn

The specifics of the variants	Ascorbic acid (mg/100 g FW)	Total carotenoids (mg/100g FW)	TPC (mg GAE/g FW)	AA (DPPH (mmol TE/100g)
V_1-\`Basin $F_1$\`-untreated	6.48 ^{d}	0.77 ^{ns}	54.48 ^{b}	141.46 ^{d}
V_2-\`Basin $F_1$\`-treated	6.71 ^{c}	0.89 ^{ns}	57.19 ^{a}	165.31 ^{b}
V_3-\`Challenger $F_1$\`- untreated	7.47 ^{b}	0.83 ^{ns}	52.71 ^{b}	155.14 ^{cd}
V_4\`Challenger $F_1$\`-treated	7.79 ^{a}	0.98 ^{ns}	57.31 ^{a}	178.05 ^{a}
LSD ($P \leq 0.05$)	0.32	0.30	3.15	20.62

The content of phenolic compounds varies with the hybrid and with the applied treatment. In the case of the \`Basin $F_1$\` hybrid, the Panoramix treatment increases the TPC content from 54.48 mg GAE/100 g FW to 57.09 mg GAE/100g FW and in the case of the \`Challenger $F_1$\` hybrid from 52.71 to 57.63 mg GAE/ 100 g FW.

Recent studies have shown positive effects of treatment with microbial biostimulators on TPC content in different horticultural plants, confirming the increase in quality and nutritional value (Ganugi et al., 2021). Thus, a significant increase in antioxidant activity and phenolic substances was observed in lettuce plants inoculated with *Rhizoglomus irregulare* compared to non-inoculated plants (Avio et al., 2017).

The antioxidant activity varies both with the hybrid and with the applied treatment (table 2). The antioxidant activity against the DPPH radical varies between 141.46 mmol TE/100 g (V_1) and 178.05 mmol TE/100 g (V_4), finding in both hybrids that the treatment with the Panoramix increased the antioxidant activity by 16.85% (\`Basin $F_1$\`) and 14.76% (\`Challenger $F_1$\`).

Among the compounds analyzed in this study, carotenoid compounds, ascorbic acid and polyphenolic compounds show strong antioxidant action. The treatment with biostimulators that induced in both hybrids the increase in the content of these compounds also induced the increase in antioxidant activity. Recent research has shown that free radicals play a causal role in the development of many diseases (inflammation, cardiovascular disease, cancer, diabetes, neurodegenerative disease, atherosclerosis) and aging processes. Sweet corn, having numerous constituents with free radical scavenging capacity, can be considered a valuable source of exogenous antioxidants important in preventing and treating diseases involving free radicals.

All investigated biochemical indices (except starch and carotene content) increase in the case of variants treated with the Panoramix biostimulator compared to the control variant. The Panoramix product stood out as a very efficient biostimulator in wheat culture, improving germination and the physiological processes involved in plant growth (Ayed et al., 2022). The interaction of plants with beneficial microorganisms can lead in the edible parts of plants to a significant accumulation of secondary metabolites which are nutritionally valuable functional compounds. Food quality modulation can be achieved by applying microbial biostimulators that induce accumulation of antioxidant compounds such as: ascorbic acid, phenolic compounds, terpenoids, carotenoids (Ganugi et al., 2021). For key quality attributes, the values may vary and significantly influenced by the hybrid as well (Soare et al., 2019).

CONCLUSIONS

The results of the study show that the application of the Panoramix biostimulator obtained from a mixture of fungi (*Trichoderma* spp.) and bacteria (*Bacillus* spp.) had a different influence at sweet corn, depending on the hybrid and the investigated biochemical indicators. Its application determined the accumulation of total soluble solids, sugar, ascorbic acid, total phenolic and antioxidant activity in variants treated with the Panoramix biostimulator compared to the control variant.

Among the tested hybrids, \`Challenger $F_1$\` recorded a better quality of sweet corn caryopses in the version treated with the Panoramix produc.

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DIVERSITY OF COLLECTED LOCAL VARIETIES IN BULGARIA ASSESSED BY USING THE NATIONAL ELECTRONIC REGISTER OF PLANT GENETIC RESOURCES DATA

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Abstract

Before the globalization the local communities relied on the available traditional food plants for their nutritional and health-related needs. They remain neglected despite their huge biological value and potential. Recent literature reports that local food systems are more resilient, sustainable, and adaptive during times of climate changes, pandemics, and conflicts. It is important to follow research strategies to support their preservation and more widespread use in future. Significant plant gene fund and knowledge has been gained through conducted expeditions in Bulgaria. In this paper, the status of collected local diversity in Bulgarian genebank is explored based on documentation of accessions and the focus is on the needs of future development. The ex situ collection has been enriched with 10,883 local accessions from cereals, vegetables, pulses, medicinal and aromatic plants. Collected materials are listed in the electronic register according to descriptor of FAO/Bioversity. The results from inventory show that the country is rich in genetic biodiversity of vegetable crops and pulses, which requires community support initiatives to preserve them also in situ/on farm.

***Key words**: plant genetic resources, collecting missions, data base, EURISCO.*

INTRODUCTION

Before the globalization and the intensification of agriculture production the local communities relied on the available traditional food plants for their nutritional and health-related needs. Nowadays they remain largely neglected despite their huge biological value and potential (Ivanova et al., 2021; Khoury et al., 2022).

Home gardens contribute to the conservation of biodiversity at the ecosystem, species and within species levels. They provide complex, multi-layered environments in which farmers can maintain large numbers of useful plant species managed in a sustainable manner over decades or even centuries. They may also provide a basis for the maintenance *in situ/on farm* of significant amounts of intra-specific genetic diversity of useful plant species (Antofie et al., 2020; Galluzzi et al., 2010; Raggi et al., 2022).

Recent literature reports that local food systems are more resilient, sustainable, and adaptive especially during times of climate changes, pandemics, and conflicts. Since traditional local varieties offer some special benefits over the commercial cultivars, it is important to follow research strategies to support their preservation and more widespread use in future (Bratu et al., 2022; Raggi et al., 2021; Stoilova et al., 2014).

Bulgaria is characterized by one of the richest countries with plant diversity in the Balkans. Significant plant gene fund and knowledge has been gained through conducted expeditions and basic research on the diversity and availability of local varieties in various regions (Krasteva et al., 2009; Knupffer, 2016; Simeonovska et al., 2013).

Collection of locally adapted traditional crop varieties is carried out by implementing the National Program for Conservation and Management of Plant Genetic Resources with the focus on their sustainable preservation and use, according to Plant Genetic Resources Strategy for Europe (ECPGR, 2021).

The main activities are: (1) Organizing expeditions in rural areas to collect samples from plant genetic resources of agricultural crops and wild crop relatives; (2) Compiling passport data and supplement genebank collections; (3) Sustainable conservation; (4) Phenotypic and genotypic characterization; (5)

Making the plant genetic resources available for exchange in accordance with the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009) and Nagoya Protocol (CBD, 2011); (6) Expanding the use of local agrobiodiversity – cooperation with research institutions, international collaboration, return the local and traditional varieties to hobby gardeners, promoting exhibitions, publications, media coverage, etc.

The aim of the study is to explore the status of collected local diversity in Bulgaria based on genebank documentation and to determine the needs of future development of collecting activities in rural areas.

MATERIALS AND METHODS

Plant genetic diversity of Bulgaria is maintained in 16 research institutes and 13 experimental stations at the Agricultural Academy, part from the Ministry of Agriculture. A total number of over 160,000 plant forms are maintained in various collections - over 100,000 field crops, over 5,000 vegetable crops, over 40,000 perennials and over 130 essential oil crops.

The Center for information and documentation of plant genetic resources at IPGR-Sadovo has been established in 1982 and completely renovated in 2021 under a project BG PLANTNET, financed by Bulgarian National Science Fund. It works according to the international documentation standard of FAO/Bioversity (2017).

The National genebank of Bulgaria, situated at IPGR-Sadovo, was built in 1984 and carries out a scientific program for the long-term preservation of germplasm with seeds under controlled conditions in accordance with the standards developed by FAO (2016).

The electronic register contains the following passport information: taxonomy, catalogue number of accessions, acquisition date, country of origin, donor of the sample, collection site, ecology-geographical data, biological status, type of storage: base collection (long-term), exchange collection (medium-term), work collection (short-term), *in vitro* and/or field collection, botanical garden, etc.

The register tools are useful for searching the areas of germplasm distribution, for monitoring, for deciding potential priority areas for conducting future collection missions.

The collecting mission activities were conducted under the methodology of Guarino et al. (2011) and in accordance with the ECPGR (2017) concept for *on farm* conservation of plant genetic resources for food and agriculture.

Surveys and interviews with the small local farmers were carried out. The generalized and systematized information on the traditional knowledge and good practices is available.

The data is published with free access in the European search catalogue for plant genetic resources EURISCO (http:eurisco.ecpgr.org).

RESULTS AND DISCUSSIONS

In Bulgaria, as in many countries, the losing of genetic resources of crops is increasing. Future progress in crop improvement and food security depends on immediate conservation of the plant genetic resources and their effective utilization by crop breeders. In this context, a great deal has been accomplished by IPGR-Sadovo in the last 15 years to safeguard the plant genetic resources which constitutes the natural heritage. However, much still remains to be done in improving the conservation strategies and upgrading the *ex situ* collections, which include a wide range of diversity (primitive varieties, landraces, weedy forms, and wild relatives).

The ecology-geographical conditions of Bulgaria have been a prerequisite for the establishment of a wide variety of plants that are used for commercial and non-commercial purposes. The seeds are usually inherited within the families, or received from neighbors from the same village or adjacent locations. Some types of local vegetables (tomatoes, peppers, watermelons, melons, onions, garlic, etc.) that are still grown have an increasing limited presence. Very often commercial varieties and hybrids are used in home gardens, but still some varieties exist which have passed from hand to hand or were populations, selected by traits.

The autochthonous economic valuable species (*Triticum durum*, *Triticum aestivum*, *Secale* *cereale*, *Avena sativa*, *Hordeum vulgare*, *Sorghum*, *Zea mays*, *Solanum lycopersicum*, *Capsicum annuum*, *Allium cepa*, *Brassica oleracea*, *Cucumis sativus*), horticultural and field, cereals and fodder, annual and perennial legumes and more than 250 species of traditional medicinal plants provide products for local consumption and domestic trade.

Currently, accessions with local origin comprise 23% of the *ex situ* collections, conserved at the gene bank in IPGR-Sadovo as the main priority. The accessions acquisitioned by expeditions are 10,883 - species and varietal diversity of landraces, including local varieties from home gardens and crop wild relatives from their natural habitats. According to the documentation the diversity of local varieties in Bulgaria is shown on Figure 1.

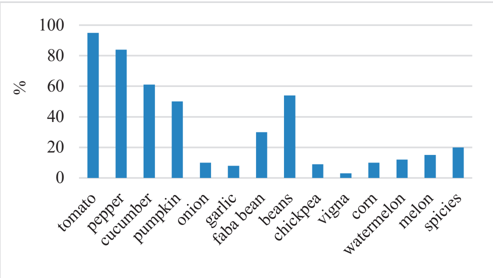

Figure 1. Diversity of local crops in Bulgaria

During the period 1982-2022 expeditions for collecting local varieties were organized in various regions of the country, funded by national and international projects. The routes of the collecting missions were determined on the basis of prior awareness of the specifics of the respective production areas or local habitats.

Villages, sufficiently distant and with different ecological and geographical characteristics were marked.

The priorities of collecting missions are: to collect variability in a particular crop or crop group; to collect tolerant forms to biotic and abiotic stress; to collect crop wild relatives, weedy types and related taxa of agricultural relevance in accordance with the reported strategies from Raggi et al. (2021, 2022).

Collected local accessions of cereals, grain legumes, vegetables and medicinal crops are a significant resource as a source material for crop breeding and for the utilization of their biological potential for healthy nutrition. These genotypes, formed as a result of the natural or artificial selection in population from farmers and adapted to a special regions in the country, get used very well to the unfavorable agri-environmental conditions.

The number of local cereal landraces and old varieties from durum wheat (118), common winter wheat (736), einkorn (67), oat (17), rye (42) and corn (250) were collected.

Grain legumes are a major contributor to global food production worldwide and are a major source of protein. They occupy about 15% of the local accessions, stored in the gene bank, represented by 45 species and the following main crops: chickpea (52), common vetch (40), latyrus (222), lupin (17), pea (29), originating from Sofia, Plovdiv, Stara Zagora, Strandzha and General Toshevo regions. The bean (2185) accessions are collected from the villages arround Smolyan, Velingrad, Kazanlak, Haskovo, Dimitrovgrad and Svilengrad.

Traditional vegetable varieties of tomato (533), pepper (1826), eggplant (56), onion crops (547), cabbages (91) and leafy vegetables (106) and spices.

The Cucurbits have been grown from thousands of years in our lands. They are of great economic importance in our country and worldwide. The fruits and seeds are used for consumption, medical purposes, as forage, as well as for decoration. The group includes *C. maxima* (65), *C. moschata* (40) and *C. pepo* (290), collected from Plovdiv and Harmanli regions.

Landraces of medicinal and aromatic plants, used in bio and home gardens for medicinal purposes, herbal teas or decoration, were collected.

The inventory of the conserved gene fund identifies areas in the country with a concentration of local varieties, where collecting missions are urgently needed to prevent the loss of valuable for crop breeding and agricultural practice diversity (Table 1).

Table 1. Localities and collected local varieties in Bulgaria during the period 1982-2022

Main crop groups	Explored regions in Bulgaria	Numb. of accessions
Cereals	Regions with extensive agriculture, including mountainous and semi-mountainous – South-East and South-West parts of the country, closed border regions, monastery lands, etc.	1,827
Grain legumes	The regions of Blagoevgrad, Kyustendil, Strandzha-Sakar, Rodopi Mountains, Ludogorie, etc.	3,099
Vegetables	Home garden regions near Gorna Oryahovitsa, Veliko Tarnovo, Svishtov, Vidin, Plovdiv, Pazardzhik, Haskovo, Dimitrovgrad, Popovo, Petrich, Sandanski, etc.	4,821
Cucurbits	Home garden regions near Pleven, Vidin, Razgrad, Shumen, Yambol, Lyubimets, Svilengrad, Ivailovgrad.	686
Medicinal and aromatic	Home gardens, monastery lands, mountainous and semi-mountainous regions.	450
Total number of collected local accessions		10,883

Ethnobotanic data related to the cultivation, utilization and genetic erosion process is recorded as done by Khoury et al. (2022).

The purposes of the development of collecting activities in rural areas are: (1) to make the traditional plant diversity available for use by current and future generations; (2) to improve the biological and economicaly important traits in crop varieties through plant breeding processes; (3) to meet the needs of farmers rural and eco communities; (4) for research activities; and (5) to restore diversity lost *in situ/on farm* and natural habitats.

These goals could be achieved through sustainable protection of traditionally grown local varieties; active participation of all stakeholders, especially inclusion of students in this process; development of target projects and establishment of a network of partners and united teams; searching for opportunities to restore the traditional varieties in districts where there is an interest using the hystorical data.

Home gardens should be considered as a model for sustainable agro-food systems, integrating both economic and ecological advantages. Some of local farmers already sell the production of vegetables and beans, as well as their products for supply guesthouses and tourist resorts with added value.

CONCLUSIONS

In the period 1982-2022 the Bulgarian genebank was enriched with rich diversity of 10,883 local varieties from cereals, grain legumes, vegetables, cucurbits, medicinal and aromatic plants.

Collected materials are listed in the National register of plant genetic resources, according to the descriptor of FAO/Bioversity and are available in EURISCO.

Genebank documentation plays significant role for conservation activities, and allows effective use of germplasm. The National register enable rapid dissemination of information to users as well as assist curators to manage the collections more efficiently.

Based on information for agro-ecological origin the distribution of local varieties from the main crop groups in Bulgaria are determined. The country is rich in genetic biodiversity of vegetables and grain legumes, which requires community support initiatives to preserve them *in situ/on farm*, through the distribution of knowledge, publicity and cooperation.

The Bulgarian home gardens can be defined as a microsystem with a high degree of diversity of plant species: vegetable crops, grain legumes, medicinal and aromatic plants, etc. still grown in different combinations in various parts of the country.

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EFFECT OF THE SEAWEED BIOSTIMULANT KELPAK® ON THE GROWTH OF CUCUMBER

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Abstract

Plant biostimulants that have a good effect on the growth and development of plants, especially when they are under the influence of stress factors. The purpose of the experiment was to study the effect of seaweed extract Kelpak® on the development of cucumbers, greenhouse production, under different forms of treatment. The experiment was carried out in the unheated glass greenhouse in the Sandanski region - a city in Southwestern Bulgaria with an altitude of 296 m, in 2019, with April planting. Biostimulant Kelpak was applied in two directions: 1) for soaking seeds and roots and 2) treatment during the growing season. 6 variants in four replicates were developed: 1) control nontreated plants – (NT); 2) treated seeds (TS); 3) treated seedling roots (TSR); 4) only vegetation treatment (VT); 5) treated seeds + vegetation treatment (TS+VT); 6) treated seedling roots + vegetation treatment (TSR+VT). Visible differences in plant growth occur after the first vegetation treatment. The variant with only treated seeds (TS) had the highest number of leaves (23rd day after transplanting), the highest number of fruits at the first harvest and the longest stem length.

Key words*:biiostimulant, cucumbers, Kelpak, seaweed, seed treatment.*

INTRODUCTION

Since the twelfth century, seaweeds, especially brown algae, have been employed in agriculture with considerable success (Temple and Bomke, 1988).

The ratio of cytokinin to auxin has an impact on morphogenesis; low amounts of cytokinin encourage the creation of roots, whereas high levels encourage the formation of buds (Stirk and van Staden, 2006).

Auxins are believed to promote root growth in a number of plants, whereas a high level of cytokinins encourages growth above ground while leaving roots in an underdeveloped state. More nutrient absorption is correlated with denser roots (Zhao, 2010).

In addition to their direct effects on plants, algal extracts may also indirectly affect plant growth. This happens during soil application, affecting microbiological activity and thus improving plant nutrition (Dhir, 2022).

Warman and Munro-Warman (1993) tested different concentrations of Maxicrop and Micro-Mist 300 and Kelpak seaweed extracts on different vegetable crops. They found that thetreatments did not lead to an improvement in vegetables yield compared to the control option, and even vice versa. The authors indicated that increasing concentrations resulted in even a slight decrease in yields.

Zamani et al. (2013), in their review, summarized that the application of seaweed extracts leads to an increase in stress resistance in most plant crops but indicated that the way in which the extracts act has a beneficial effect on plants.

Bulgari et al. (2019) add that the impact of biostimulants (positive or negative) on vegetable plants in order to improve their resistance against abiotic stress, is the result of many constituent elements. They summarize that the mode of action is understood by the way the plants react, but not only at the physiological level, but also at a deeper level (molecular and biochemical level).

Application of seaweed concentrate (root treatment or foliar spray) has been found to improve cabbage seedling growth (Aldworth and Van Staden, 1987).

El-Gamal et al. (2020) found that treating faba bean with seaweed extracts counteracted drought stress.

Three different seaweed biostimulants were tested on small-fruited tomatoes, applied by foliar spraying three times, and with all three

preparations, the early maturity of fruiting and the number of fruits were improved. Mikiciuk, M., & Dobromilska, R. (2014).
A biostimulant called Kelpak is created from the seaweed Ecklonia maxima. Auxins, cytokinins, polyamines, gibberellins, abscisic acid, brassinosteroids, and phlorotannins like phloroglucinol and ecol are among the plant growth regulators found in it. A stronger, healthier plant produces better yields as a result of the combined actions of these substances (Rengasamy et al., 2015).
In a trial of different applications of Kelpak on pepper, it was found that pre-planting seedling immersion in the solution, followed by a three-time vegetative spray, resulted in an increase in fruit number and size (Arthur et al., 2003).
When testing the application of two seaweed extracts on cucumbers, in combination with a low temperature treatment, it was found that the combination of the application of low temperatures for 5 days and the treatment with seaweed extracts had the greatest effect on the flowering of cucumbers (seaforce + seamino). A significant increase in cucumber yield was obtained when treated only with the extracts (seaforce + seamino) from seaweed (Sarhan & Ismael, 2014).
Valencia et al. (2018) proved that foliar treatment of cucumbers with five different seaweed extracts applied as biofertilizers led to improvements in both growth and fruit yield and quality.
In a fertigation applicability test of the seaweed biostimulant True Algae Max (TAM) used in different ratios and combined with NPK (100%) it was found that the extract (applied alone and in combination with NPK) resulted in an increase in cucumber yields (Hassan et al., 2021). In conclusion, the authors conclude that the use of mineral fertilizers can be avoided when applying this biostimulant.
Treatment of zucchini grown in a saline environment with different concentrations of Ecklonia maxima seaweed extract applied as a foliar spray resulted in overcoming stress and increasing plant biomass and yield (Rouphael et al., 2017).
Cristofano, et al. (2021) in their review examining the impact of different biostimulants on Cucurbitaceae, Solanaceae and leafy vegetables summarized that, in general, biostimulants have proven their benefits in improving plant development and increasing yields. But because there are a large number of factors also influencing and controversies taking shape, they conclude that research into the wide variety of biostimulants produced by different types of kelp should continue, broadening the scope and using an interdisciplinary approach. approach.
The purpose of the experiment was to study the effect of seaweed extract Kelpak® on the development of cucumbers, greenhouse production, under different forms of treatment.

MATERIALS AND METHODS

The experiment was carried out in Sandanski. The town of Sandanski (224 m above sea level) is located in the Sandanski-Petrich valley, at the foot of the Pirin Mountains.
The basin has a transitional Mediterranean climate and is one of the warmest places in Bulgaria. The average monthly temperature is the lowest in the month of January and is positive, and the highest is in the month of July. During the experimental period, the average daily temperature for March was 12.5°C, and although it was about +3°C warmer (for a multi-year period), a sharp decrease in minimum temperatures was recorded twice during seedling production. April has temperatures close to normal for this period of the year (14.5°C), only +0.2°C above the norm for a multi-year period. May is cooler compared to average data from previous years (-0.8°C), with an average daily temperature of 17.8°C. June, like April, has temperatures close to normal (only by +0.1°C) for this period of the year (Figure 1).
Precipitation also does not directly affect the growth of plants in cultivation facilities, but like temperature it indirectly affects the micro-climate in greenhouses. March is generally dry with only 4 rainy days and with a total amount of precipitation of approximately 1 mm. 13 rainy days were reported in April and 10 rainy days in May. June has 8 rainy days (Figure 1). .

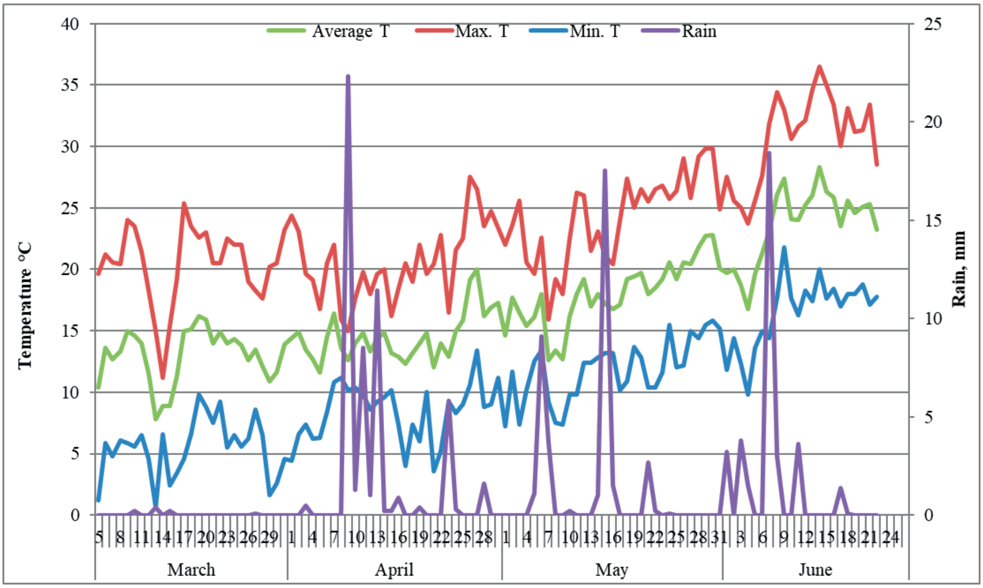

Figure 1. Meteorological situation in the town of Sandanski during the experimental period, 2019

Table 1. Content of phytohormones in biostimulant Kelpak®

Type	Quantity	Unit
Auxins	10.7	µg/L
Cytokinins	0.03	µg/L
Gibberellins	0.6	µg/L
Brassinosteroids	1.1	µg/L
Polyamines	4000	µg/L
Phlorotannins	2200	µg/L

The experiment was set up in an unheated glass greenhouse with a shed width of 3 m. In the month of April, three rows of cucumbers were planted in each shed, with a distance between rows of 110 cm.

For the purposes of the experiment, the variety Defense F1 (Enza Zaden), suitable for summer-autumn production in greenhouses, was used. The hybrid has a vigorous growth, with dark green fruits 30-33 cm long

Biostimulant Kelpak® is a 100% extract of the seaweed Ecklonia maxima (kelp), with a high content of plant hormones (Table 1).

It was applied in two directions: for seed and root soaking and treatment during the growing season, and 6 variants were developed in four replications: 1) control nontreated plants – (NT); 2) treated seeds (TS); 3) treated seedling roots (TSR); 4) only vegetation treatment (VT); 5) treated seeds + vegetation treatment (TS+VT); 6) treated seedling roots + vegetation treatment (TSR+VT).

The dilution of the preparation is 1:100, with a treatment rate of 250 ml/day. Before planting, the seeds are soaked for 6 hours.

Cucumber seedlings obtained from untreated seeds were subjected to pre-planting (TSR) root soaking. Plant roots were soaked in the solution for 10 minutes, immediately before transplanting, until the substrate was completely moistened. The seedling trays were slightly drained of the excess solution, and transplanting in the greenhouse was started.

Planting was carried out on April 15, 2019, with each variant including 90 plants planted 50 cm apart in the row.

Two vegetative treatments were carried out by foliar spray of the cucumbers. The first treatment was carried out on April 28, 2019 (14 days after transplanting), and the second after another 14 days, on 13.05. 2019, as a guideline,

vegetation treatments should be completed before the plants flower.
All elements of the agrotechnics of the experience (tillage, fertilization, irrigation, etc.) are the same for all variants. A ribbon drip hose was used for drip irrigation.
During the growing season of the plants, phenological observations were made: germination, third-fourth leaf, one week after the first vegetation treatment, beginning of flowering, beginning of fruiting. Phenological observations were carried out according to variants.
Biometric parameters were established in four replications of 10 marked plants. The measurements were made when the produce was harvested. The following indicators were tracked: height of plants; average number of leaves; fruit length and diameter; average weight of one fruit; yield

RESULTS AND DISCUSSIONS

Observations on the growth and development of plants begin with the establishment of the experiment.
The beginning of germination started on the fourth day after sowing the seeds, and no differences were reported between the variants. Seven days after sowing the seeds (March 13, 2019), mass germination (97%) was recorded, again with no differences between the individual variants.
In the 3rd/5th leaf phase (29.04.2019), which is 14 days after transplanting the plants, the first vegetative treatment with Kelpak® was carried out. Until this phase occurs, there are no visible differences in the growth of the plants; therefore, the treatment was carried out in one day.
Nine days after the 1st vegetative treatment, leaves were counted to determine if there were differences between the variants. The difference is not great. The variant with treated seeds (TS) has more leaves - on average 10 per plant, the difference with the other variants being 1-2 leaves.
The number of leaves of the plants by variants was counted before the second vegetation treatment and before the first harvest. In these two readings, it was found that the remaining variants differed in number of leaves from the variant with only treated seeds. In TS, the formation of leaves is delayed and at the last reading (41st day after transplanting) the average number of leaves is 30, while in the variants with three treatments (TS+VT and TSR+VT) the number of leaves is the highest and they have an average of 35 leaves. At the last reading of the number of leaves, the difference between the other variants is on an average 1-2 leaves.
A second foliar spray with Kelpak® was made, 14 days after the first treatment (13.05.2019), which is before the flowering of the plants. A few days later, the beginning of flowering was reported, with almost no differences between the individual variants. The differences are mainly in the number of plants with flowers, but the beginning of flowering is observed in almost all variants.
Only the last two variants, with treated seeds and vegetation treatment (TS+VT), as well as treated roots + vegetation treatment (TSR+VT), delayed the setting of flowers.
Start of fruiting: In order to realize sufficient production, the first harvest was carried out simultaneously from all variants on 25.05.19. The difference between the variants was not in the timing but in the amount of fruit picked.
At the first harvest, the variant with treated seeds, which produced the most fruits and, accordingly, the highest yield for this harvest, is selected again.

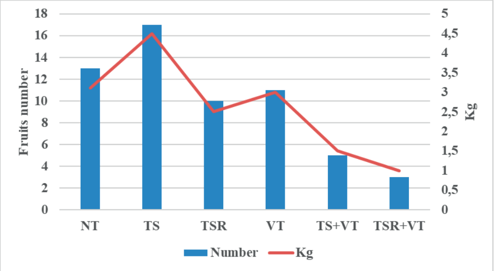

Figure 2. Number of fruits and yield of first harvest of cucumbers

The least fruits were obtained from the last two variants: TS+VT and TSR+VT, which are also due to the delayed entry into the generative phase (Figure 2).
During the harvesting period, biometric measurements of the plants were made.
Of the treated variants, the plants with the highest height were from the variant with only

treated seeds (265 cm). In the rest of the treated variants, a decrease in the height of the plants was noticed compared to the control, and the plants of the variant with treated roots were the lowest (251 cm). In the variants with vegetation treatment, by spraying the leaves, the heights are almost equal, but again, the one with the smallest visibility is the one in which soaking of the roots was also applied (TSR+VT).
This indicates that it is likely that the application of Kelpak®, by soaking the roots, leads to a reduction in plant height.
The biometric measurements of the fruit parameters show small differences in the length of the fruits, while in diameter they are almost the same.
The length of the fruits is in the range of 26.5 to 28.7 cm, and in almost all treated variants, the fruits are of a smaller length (26.5-27 cm) compared to the control (28 cm). Only in the variant with treated seeds and two vegetation feedings are the fruits of greater length (28.7 cm).
As for the diameter, in all variants it moves within narrow limits (3.3-3.5 cm).
The average weight of the fruits ranges from 231 to 242 g. Fruits in all variants with vegetation treatments were heavier than those in the control, while in variants without vegetation treatments they were lighter.
The variant with the smallest average weight of the fruit is the one with treated roots (231 g), and the heaviest are the fruits of the variant with treated seeds + vegetation treatments (242 g).
When comparing the varieties with single treatments before planting, the seed-treated variety had a greater mean weight, although the fruits were on average 2 g lighter than those of the control. And of the variants with the vegetation treatment, the one with the largest fruit mass is again the one in which the seed treatment was also applied (TS+VT).
This indicates that it is likely that the treatment of seeds with Kelpak® leads to an increase in fruit mass.
Based on the realized harvests, it was found that almost all variants treated with Kelpak® gave lower yields in percentage ratio than the control (Figure 3). Only in the variant with only treated seeds, the yield in percentage terms is slightly higher than in the control, which is in confirmation of the results obtained from Warman and Munro-Warman (1993).

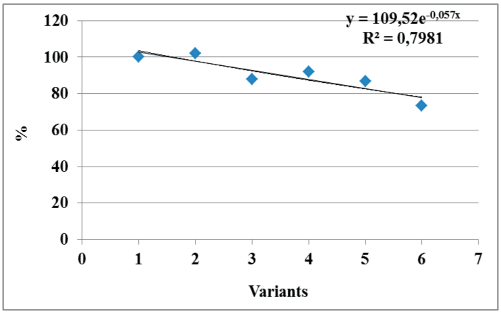

Figure 3. Yield of cucumbers in %

Although in the variant with treated seeds + vegetation treatments, the fruits are the heaviest, due to the smaller number of fruits, a low yield is also obtained. In the variant with only treated seeds, the fruits had a smaller mass than those of the control, but the greater number of fruits obtained in this variant led to higher yields compared to the control (Figure 3).
Based on the realized harvests, it can be concluded that the one-time treatment of only seeds leads to slightly higher yields.

CONCLUSIONS

Several conclusions can be drawn from the obtained results and the performed analyses:
A single treatment of the seeds, by soaking them without additional vegetation treatments with Kelpak® solution, leads to a better overall development of the plants. They are taller and have more leaves. Already at the first harvest, a larger number of fruits was obtained, and this trend was maintained until the last realized harvest.
When the treatment of the seeds was added to the treatment during the growing season by spraying the plants, there was a delay in their development. Despite the highest average weight of the fruits, affected by the delayed development and the small number of fruits, the lowest yield was obtained until the last realized harvest.
When treating the roots of the seedlings, there was a delay in the development of the plants, which also led to low yields.

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DYNAMICS OF NUTRIENT ELEMENTS IN THE SOIL IN THE CULTIVATION OF DIFFERENT RASPBERRY GENOTYPES

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Abstract

The present study follows the dynamics of nutrient elements in the soil of a raspberry plantation during the period of blossoming and fruit harvesting. The scientific experiment was conducted in the period 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan with four raspberry genotypes, such as Willamette, Meeker, Samodiva and Magdalena. The soils of the region are gray forest, moderately eroded, low in humus and prone to waterlogging.The content of basic nutrients (nitrogen, phosphorus, potassium), humus and pH in the 0-20 cm and 20-40 cm soil layers was analyzed. The results show that the highest nitrogen content was registered the surface soil layer with 89.77 mg/kg during the blossoming phenophase in 2019.The amount of phosphorus varied widely 2.60-6.20 mg/100 g. The highest potassium content (28.66 mg/100g) was found again in the surface soil layer during blossoming of the 2020 raspberry plants.

Key words: *raspberries, varieties, soils, agrotechnics, nutritional elements.*

INTRODUCTION

Raspberry (*Rubusidaeus* L.) is a fruit species that is widespread on almost all continents because of its plasticity and taste of the fruit. It is widely used in the foot-hill and mountain regions in Bulgaria, such as Troyan, Teteven, Berkovitsa, where soil and climate conditions are largely favorable for its development. In these places, the climate is cool, and soil and atmospheric humidity are higher.

The fruit agrocenosis suffers active anthropogenic impact, which is mainly aimed at increasing yield. At the same time, changes in soil fertility indicators (amount and content of organic matter, activity of accumulation and migration of substances, including nitrate compounds and water-soluble salts) determine soil sustainability as a biosphere component (Maliuk et al., 2020).

Every crop needs an optimal nutrient balance in the soil for good plant development. Obtaining economically justified yields is possible only by providing conditions for balanced nutrition and development of raspberry plantations (Kljajic, 2017). Determining the agrochemical composition of soil profiles is extremely important when growing fruit crops and providing them with nutrients, especially nitrogen, phosphorus and potassium, which is a major task in agriculture.

Until now, the research into the soil-plant system has been very limited in the Bulgarian raspberry production, and the literature lacks data on raspberry plantations on gray forest soils in mountaine regions (Petkova et al., 2013). The most important nutrients for raspberries are nitrogen and potassium (Fiedler, 1970; Bergman, 1988; Smolarz, 1999). Nitrogen stimulates vegetative growth, and potassium affects plant yield, increases drought tolerance and improves cold tolerance of raspberries (Motosugi et al., 1995; Németh et al., 2002).

Canadian scientists found that the content of nitrogen and potassium in ripe fruits is eight times greater than the content of phosphorus, calcium and magnesium (Kowalenko, 1994). This proves that raspberries need nitrogen and potassium more than phosphorus. Rempel et al.

(2004) and Kowalenko (2006) found that the optimal nitrogen amount in the soil stimulated the growth of raspberry shoots in length andthickness. In 2009, Zlatareva & Nikolov found that fertilizer rates higher than 12 kg N/da in a fruit-bearing plantation provide better nutrition for the raspberry plant.
The aim of the present study is to trace the content of the main nutrients (nitrogen, phosphorus, potassium), humus and pH in the soil of a raspberry plantation at different plant phenophases.

MATERIALS AND METHODS

The scientific experiment was conducted in the period 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The objective of the study are four raspberry genotypes, such as Willamette, Meeker, Samodiva and the candidate cultivar Magdalena, that has been selected in RIMSA - Troyan. The area is maintained in black fallow in the intra-row spacing, whereas the inter-row spacings are naturally grassed, with the application of the appropriate mowing of the grass. Fertilizing is carried out in the intra-row area to achieve optimal values of the individual nutrients for the raspberry crop.The following fertilizing was applied for the three experimental years: 20 kg/da ammonium nitrate (annually), 15 kg/da triple superphosphate and 7.5 kg/da potassium chloride (first and third year). The plants are grown under irrigated conditions with drip irrigation.
The soils in the region are gray forest, medium to heavy sandy-clay, moderately eroded with low humus content (Mihailova et al., 2008), poor in digestible phosphorus and potassium and with a high absorption capacity in relation to phosphorus (Nikolov, 1969; Nikolov, 1985; Mihailova et al., 2006). Gray forest soils are distinguished by a low content of total nitrogen, which slightly varies under the influence of fertilizing with both nitrogen and phosphorus mineral fertilizers (Petkova et al., 2013) and poor in humus (about 2% in plowed areas). Most of the organic matter is concentrated in the uppermost part of the humus horizon, where there is also the greatest concentration of soil microflora and fauna (Valchovski, 2010).
The stock of soil with the main nutrients was determined:
Soil reaction (pH) - determined potentiometrically.
Nitrogen (mg/kg) - according to the Bremner and Keeney method in 2018 and according to the Kjeldahl method, BDS - EN ISO 5983, in 2019 and 2020.
Phosphorus (mg/100 g) - according to the method of P. Ivanov
Potassium (mg/100 g) - according to the method of P. Ivanov
Humus (%) - according to Tyurin
The mechanical composition of the soil was determined by Rutkowski method.
The data were processed according to the methods of two-factor variance (ANOVA) and descriptive analysis (Lidanski, 1988), the software product MS Excel - 2010 was used.

RESULTS AND DISCUSSIONS

As a result of the performed analyzes it was established that the variety of the soil by mechanical composition is heavily sandy-clayey to clayey (with physical clay content from 30 to 90%), moderately eroded with low humus content (Table 1).

Table 1. Mechanical composition of the soil, determined by the method of Rutkowski (1972)

Cultivars	Soil layers / Soil type / Physical clay content, %		
	0-20 cm	20-40 cm	40-60 cm
Willamette - 0.50 m	Clay (60)	Clay (60)	Clay (90)
Willamette - 0.30 m	Clay (48)	Clay (42)	Clay (78)
Meeker - 0.50 m	Clay (60)	Heavy sandy-clay (30)	Clay (36)
Meeker - 0.30 m	Clay (54)	Clay (48)	Clay (60)
Samodiva - 0.50 m	Clay (60)	Clay (66)	Clay (80)
Samodiva - 0.30 m	Clay (78)	Clay (60)	Heavy sandy-clay (30)
candidate cultivar Magralena - 0.50 m	Clay (36)	Clay (60)	Clay (54)
candidate cultivar Magralena - 0.30 m	Clay (54)	Clay (54)	Heavy sandy-clay (30)

The agrochemical analysis for the content of basic nutrients (N, P, K), humus and pH was reported during the period of blossoming and fruit harvesting in two soil layers from the intra-row spacing of the plantation.

Table 2. Composition of agrochemical indicators from the soil layers of the raspberry plantation for 2018

Soil layers cm		pH		∑ N- NH_4+NO_3	P_2O_5	K_2O	Humus
		H_2O	KCl	mg/kg	mg/100 g		%
	During blossoming						
0-20 cm	Minimum	5.00	4.40	14.40	2.60	10.00	0.81
	Maximum	5.00	4.50	34.60	8.30	16.90	1.44
	Mean	5.00	4.47	27.67	5.37	12.63	1.15
	St error	0.00	0.03	6.64	1.65	2.15	0.18
	St Dev	0.00	0.06	11.49	2.85	3.73	0.32
	CV%	0.00	1.34	41.53	53.07	29.53	27.83
20-40 cm	Minimum	4.90	4.30	15.00	1.70	8.00	0.26
	Maximum	5.00	4.50	31.70	10.00	18.20	1.71
	Mean	4.97	4.37	21.90	6.20	12.87	0.92
	St error	0.03	0.07	5.03	2.42	2.95	0.42
	St Dev	0.06	0.12	8.72	4.19	5.12	0.73
	CV%	1.21	2.75	39.82	67.58	39.78	79.35
	During fruit harvesting						
0-20 cm	Minimum	4.40	3.80	34.60	1.40	6.20	0.90
	Maximum	5.20	4.60	49.50	7.60	14.80	1.64
	Mean	4.76	4.20	43.64	3.26	10.16	1.20
	St error	0.17	0.17	3.09	1.12	1.42	0.14
	St Dev	0.38	0.38	6.91	2.51	3.17	0.32
	CV%	7.98	9.04	15.83	76.99	31.20	26.67
20-40 cm	Minimum	4.60	3.90	31.70	0.50	5.30	1.06
	Maximum	4.90	4.40	69.10	4.40	10.40	1.37
	Mean	4.78	4.12	41.80	2.60	8.00	1.18
	St error	0.05	0.09	6.89	0.83	0.96	0.05
	St Dev	0.11	0.19	15.40	1.85	2.15	0.11
	CV%	2.30	4.61	36.84	71.15	26.88	9.32

In 2018, during the raspberry blossoming phenophase, the soil acidity was defined as highly acidic, with a pH range of 4.97 (20-40 cm) to 5.0 (0-20 cm) (Table 2). The average nitrogen content recorded in the surface layer (0-20 cm) was 27.67 mg/kg, and at a depth of 20-40 cm it was slightly lower at 21.90 mg/kg, values indicating low stocking. The average values of phosphorus during the phenophase of full blossoming of raspberries in the upper soil layer are respectively 5.37 mg/100g. The phosphorus content is significantly higher in the next soil layer (20-40 cm), where it reaches values of 6.2 mg/100 g, which is defined as an average stock. The concentration of potassium at the two soil depths is 12.63 and 12.87 mg/100 g, respectively. The reported results for the humus content show that its values from the soil profiles are low - 1.15% at 0-20 cm and 0.92% at 20-40 cm (Table 2).

During the 2018 raspberry harvest, the soil reaction at the 0-20 cm soil layer was 4.76, and at the 20-40 cm depth it was 4.78 and was characterized as highly acidic (Table 2). The average nitrogen content recorded in the surface layer is 43.64 mg/kg., and in the lower layer 41.8 mg/kg, which defines the soil as moderately stocked with the element. Phosphorus values during the phenophase of raspberry harvesting in the upper soil layer are respectively 3.26 mg/100 g, in the next it significantly decreases to reach an average value of 2.60 mg/100 g. During the phenophase of fruit harvesting, a low potassium content ranging from 10.16 mg/100 g (0-20 cm) to 8.0 mg/100 g (20-40 cm) was recorded.The content of humus in the upper soil layers is low - 1.2% and 1.18%

Table 3. Composition of agrochemical indicators from the soil layers of the raspberry plantation in 2019

Soil layers cm		Active reaction pH	Total mineral nitrogen	P_2O_5	K_2O	Humus
		H_2O	mg/kg	mg/100 g		%
	During blossoming					
0-20 cm	Minimum	4.56	83.81	2.62	22.09	2.17
	Maximum	5.36	96.06	4.73	34.15	2.77
	Mean	5.03	89.77	3.34	28.53	2.39
	St error	0.09	1.53	0.23	1.30	0.07
	St Dev	0.25	4.33	0.66	3.69	0.19
	CV%	5.03	4.83	19.70	12.93	7.82
20-40 cm	Minimum	4.69	77.35	2.18	21.9	2.03
	Maximum	5.2	94.97	3.98	31.88	2.86
	Mean	4.99	83.53	2.86	27.13	2.36
	St error	0.07	2.28	0.23	1.15	0.09
	St Dev	0.20	6.46	0.66	3.24	0.27
	CV%	3.93	7.73	22.97	11.95	11.25
	During fruit harvesting					
0-20 cm	Minimum	5.51	60.97	4.78	20.67	1.86
	Maximum	5.88	69.05	7.16	22.91	2.01
	Mean	5.67	64.47	6.06	21.95	1.96
	St error	0.08	1.71	0.49	0.52	0.03
	St Dev	0.17	3.42	0.98	1.04	0.07
	CV%	2.94	5.31	16.23	4.76	3.51
20-40 cm	Minimum	5.59	59.73	4.22	19.78	1.79
	Maximum	5.98	62.32	6.5	20.46	1.94
	Mean	5.74	60.98	5.19	20.07	1.87
	St error	0.09	0.55	0.48	0.15	0.04
	St Dev	0.17	1.10	0.96	0.29	0.07
	CV%	2.99	1.81	18.56	1.46	3.94

In 2019, in the blossoming phenophase, both investigated soil layers had a pH of 5.03 (0-20 cm) and 4.69 (20-40 cm) (Table 3), which are defined as - highly acidic. The reported amount of total nitrogen averaged 89.77 mg/kg (0-20 cm) and 83.53 mg/kg (20-40 cm).

The analysis shows that the nitrogen content is significantly higher than the previous year, recorded in the same phenophase and soil layers.

The average values of phosphorus during the blossoming phenophase of raspberries in the upper soil layer are respectively 3.34 mg/100 g, and in the next layer they are 2.86 mg/100 g, which is defined as low stocking. The results regarding potassium content show that both soil depths are well stocked with the nutrient, 28.53 mg/100 g at 0-20 cm and 27.13 mg/100 g at 20-40 cm, respectively. The content of humus in the two soil layers is almost equal (2.36-2.39%), but it is not satisfactory for the needs of the plants.

During the raspberry harvest (2019), the soil reaction ranges between 5.67-5.74, which characterizes it as moderately acidic (Table 3). The nitrogen content in the soil ranges from 60.98-64.47 mg/kg, which is medium to good stocking, although the nitrogen content decreases with depth. During fruit harvesting, the phosphorus content increased almost twice and was within the limits of 6.06 mg/100 g (0-20 cm) and 5.19 mg/100 g (20-40 cm).

The results regarding potassium content show that both soil depths are well stocked. At 0-20 cm it was 21.95 mg/100 g and at 20-40 cm it was 20.07 mg/100 g, but a slight decrease of the element was observed compared to the blossoming phenophase. The humus content varies slightly, with 1.96% in the top soil layer and 1.87% in the next soil layer.

Table 4. Agrochemical indicators from the soil layers of the raspberry plantation in 2020

Soil layers cm		Active reaction pH H_2O	Total mineral nitrogen mg/kg	P_2O_5 mg/100 g	K_2O mg/100 g	Humus %
	During blossoming					
0-20 cm	Minimum	4.07	76.39	3.88	25.42	1.98
	Maximum	5.02	96.11	5.95	32.62	2.44
	Mean	4.52	88.03	4.56	28.66	2.20
	St error	0.13	2.33	0.25	0.79	0.05
	St Dev	0.37	6.58	0.70	2.24	0.15
	CV%	8.12	7.48	15.29	7.81	7.05
20-40 cm	Minimum	3.91	74.59	3.35	23.79	1.94
	Maximum	4.91	93.34	4.98	31.09	2.51
	Mean	4.34	85.03	4.19	27.68	2.22
	St error	0.13	2.25	0.20	0.74	0.07
	St Dev	0.38	6.37	0.56	2.08	0.18
	CV%	8.69	7.50	13.35	7.52	8.31
	During fruit harvesting					
0-20 cm	Minimum	4.77	61.61	3.86	25.86	1.98
	Maximum	5.51	74.00	5.18	26.73	2.06
	Mean	5.14	67.81	4.52	26.30	2.02
	St error	0.37	6.20	0.66	0.44	0.04
	St Dev	0.52	8.76	0.93	0.62	0.06
	CV%	10.18	12.92	20.65	2.34	2.80
20-40 cm	Minimum	4.45	59.41	3.75	24.8	1.75
	Maximum	5.35	72.09	4.25	25.42	2.00
	Mean	4.9	65.75	4.00	25.11	1.88
	St error	0.45	6.34	0.25	0.31	0.13
	St Dev	0.64	8.97	0.35	0.44	0.18
	CV%	12.99	13.64	8.84	1.75	9.43

The analysis of the results of the study of the agrochemical status of the soil in the 2020 experimental year during the full blossoming phenophase show that the soil reaction is strongly acidic - 4.52 at 0-20 cm and 4.34 at 20-40 cm (Table 4).

Total mineral nitrogen is high at both depths. In the first (0-20 cm) it is 88.03 mg/kg and in the second (20-40 cm) it is 85.03 mg/kg. The phosphorus content is higher in the first soil layer - 4.56 mg/100 g, with a tendency to decrease in depth. The results regarding potassium content show that both soil depths are well stocked. At 0-20 cm it is respectively 28.66 mg/100 g and at 20-40 cm it is 27.68 mg/100 g.

The content of organic matter in both soil layers is low (from 2.20% to 2.22%). The analysis of the results during fruit harvesting show that the active reaction of the soil is from highly acidic - 4.9 at 20-40 cm, to moderately acidic 5.14 from 20-40 cm (Table 4).

The reported total mineral nitrogen has lower values compared to the full blossoming phenophase. At the first depth, the element is within the limits - 67.81 mg/kg, and at the second depth - 65.75 mg/kg. These values show that the soil is well stocked with nitrogen and can satisfy the plants' needs. During the fruit harvesting, the phosphorus content was kept within close limits of 4.52 mg/100 g (0-20 cm) and 4.00 mg/100 g (20-40cm). The results regarding potassium content show that both soil depths are well stocked, but a slight decrease in the element was observed compared to the blossoming phenophase. The degree of variation is very low CV% - 2.34% (0-20 cm), CV% - 1.75% (20-40 cm). The content of humus is again very low - 2.02% in the upper soil layer and 1.88% in the lower soil layer. This content of organic matter is unsatisfactory for plants.

CONCLUSIONS

Based on the analyzes of the soil profiles from different depths and periods (blossoming period and fruit harvesting period), it was established that they have different reserves of nitrogen, phosphorus and potassium.

The studied soil layers of 0-20 cm and 20-40 cm of the intra-row spacing show a strongly to moderately acidic reaction in aqueous solution.

Nitrogen content in the first year varies from low during the blossoming period to moderate during the fruit harvesting period. Over the next two years, nitrogen values ranged from good during the blossoming phenophase to very well stocked during the fruiting period.

The amount of phosphorus is in the range from low to medium stock in the soil depths.

The analyzed soil layers in 2018 are poorly stocked with potassium. In the following two years, a good supply of the element was registered with values above 20 mg/100 g.

The organic matter in all three years of the experiment was low in quantities unsatisfactory for the needs of the raspberry plants.

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PRELIMINARY EVALUATION ON GETTING SPROUTS IN SORREL ON DIFFERENT SUBSTRATES

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Abstract

In recent years, there is an increasing trend worldwide in consuming microplants (microgreens / sprouts). Due to their benefits for human health, less growing requirements, high nutritional composition and their versatility in various cuisines, microplants become of great interest. Therefore, there is a need in finding sustainable possibilities for obtaining such sources, rich in health-promoting compounds. Thereby, the objective of this paper is to present the results of an experimental design that involves the obtaining of Rumex acetosa L. (sorrel) sprouts (microplants) on different cultivation substrates. The biological material consisted of seeds of sorrel, and six different substrates varying the ratio between perlite, banana peel and agar. The experiments were performed in a non-aseptic system. After 14 days, the seed germination capacity and the number of leaves were recorded. Also, several physicochemical and biochemical analyses performed for the resulting sprouts (microplants), such as micro and macronutrients content, assimilatory pigments and dry matter (DM). The most promising cultivation substrates for sorrel sprouts (microplants) are perlite and the mixtures of perlite and agar. In terms of sprouts quality, both variant 2 (perlite 75% with agar 25%) and variant 4 (perlite 25% with agar 75%) of cultivation were highlighted.

Key words*: cultivation substrates, germination, nutrients, quality of sprouts (microplants), sorrel.*

INTRODUCTION

In recent years, with the increased interest in healthy eating, society has turned its attention to fresh or functional foods, such as small-scale vegetables microplants, called "vegetable confetti" (Bhaswant et al., 2023). Microplants, both sprouts and microgreens, have higher nutritional value than mature vegetables or seeds (e.g., vitamins, chlorophyll, macro and microelements), reveal exciting flavours and tastes, and can be quickly cultivated under the influence of LEDs artificial light, being more and more diversified and more often found on supermarket shelfs (Zhang et al., 2021).

One of the plants that claim to be produced as sprouts is sorrel (*Rumex acetosa* L.) (Choe et al., 2018). Sorrel is a green plant with spontaneous growth that belongs to the *Polygonaceae* family (Li et al., 2022). This is a fast-growing perennial plant, resistant to various abiotic factors (e.g., low temperatures in winter) and biotic (diseases or pests). The leaves or stems of *Rumex* are harvested for fresh consumption and used as raw in salads or for various culinary preparations, bringing a nutritional contribution to a balanced diet. (Spínola et al., 2018; Barbu et al., 2021). Unfortunately, it has a high oxalic acid content, which can harm health if ingested in large doses, affecting the kidneys and renal tubule (Farré et al., 1989). However, sorrel eaten in moderation prevents and controls some diseases. Seeds, leaves, stems, or roots are used worldwide as "drugs" in alternative medicine (Bello et al., 2019). As a medicinal plant, sorrel (*Rumex acetosa* L.) can treat gastrointestinal disorders and skin diseases, improve eyesight, stabilize blood pressure, and prevent scurvy (Barbu et al., 2021). In addition to the nutritional properties, sprouts involve minimal resources such as limited space, various nutrient substrates (e.g., peat, coconut coir, composted organic wastes, vermiculite, perlite), limited water, and zero use of pesticides (Pascual et al., 2018).

The aim of the present study was to assess the preliminary results regarding non-aseptic system for sorrel sprouts (microplants) production in controlled conditions, under LEDs light. The method is an ecological and economical option because it uses organic residues (banana peels), with an essential nutritional supply for the sprouts (sprouts). The obtained sorrel sprouts (microplants) were quantitatively and qualitatively analysed in order to highlight the most appropriate cultivation substrate for this production system.

MATERIALS AND METHODS

Biological material

The biological material consisted of sorrel (*Rumex acetosa* L.) organic certified seeds (ecological and untreated) from the commercial company Italian Sprout Srl, Italy.

The experimental design

In order to obtain the sprouts (microplants), sorrel seeds were grown *in vitro*. The experiments were carried out on five types of cultivation substrates, which combined several solid components such as perlite, agar, and banana peels with distilled water, as liquid component (Table 1).

Each experimental variant was performed in 3 replicates, with 10 glass containers each.

The seeds were sown in glass containers and placed in a cultivation chamber at 21°C ± 2°C temperature, in non-aseptic conditions and exposed to 16/8 h photoperiod artificial LEDs lights (Lee et al., 2014, Enache & Livadariu, 2016) for 13 days at an average luminous flux of 1140 lm.

Table 1. Experimental variants used in the study

Experimental variants	Composition of the nutrient substrate
V1 (CONTROL)	perlite 100%
V2	perlite 75% agar 25%
V3	perlite 50% agar 50%
V4	perlite 25% agar 75%
V5	perlite 75% organic banana peels 25%
V6	perlite 50% organic banana peels 50%
V7	perlite 25% organic banana peels 75%

Methods of analysis

1. Seed germination capacity

The germination capacity (GC%) was determined by counting the number of germinated seeds in glass containers *per* experimental variants. The results are calculated in percentages using the formula developed by Fredrick et al. (2015):

$$GC(\%) = \frac{n_g}{n_t} \times 100$$

n_g = the number of germinated seeds,
n_t = the total number of seeds

2. The number of leaves

Morphometric measurements of the sprouts consisted of counting the number of leaves per microplant using EPSON Model expression 11000XL scanner which uses the image analyser software WinFOLIATM. The results represent the average number of leaves.

3. Dry matter content (DM)

The dry matter (DM) content was determined according to the European Pharmacopoeia 7th edition methods (2010).

4. Assimilatory pigments

Assimilatory pigments content was performed using an adapted method of Wellburn (1994).

The obtained results were expressed as mg chlorophyll a (Chl a), b (Chl b), and total chlorophyll (Chl T) per g fresh weight (mg/g FW).

5. Micro and macronutrients analysis

The fresh sorrel sprouts samples were grinded and three samples were subjected to microwave extraction, as follows: 0.250 g, 8 ml HNO_3 and 2 ml H_2O_2. The control consisted of 8 ml HNO_3 and 2 ml H_2O_2. The samples were subjected to

ICP-MS analysis Agilent 7700X (Bashdar & Rasul, 2023). The analysis was realised based on the calibration curve of the multi-element standard Agilent Technologies solution.

The results of the quantitative determinations are presented as the average of at least 3 determinations accompanied by the standard deviation.

The comparison between the experimental variants was evaluated by one-way ANOVA.

RESULTS AND DISCUSSIONS

Preliminary results regarding sorrel sprouts (microplants) production, on different growing substrates based on perlite, agar and banana peels, in non-aseptic system (environment), are presented as follows.

1. Results on GC

The effect of the cultivation substrate on the germination capacity of sorrel seeds is presented in Table 2.

Table 2. Germination capacity values

Experimental variants	GC % ± SD
V1	89.33 ± 3.05 [a]
V2	86.67 ± 1.52 [ab]
V3	84.00 ± 2.00 [ab]
V4	83.33 ± 1.52 [b]
V5	5.33 ± 1.15 [c]
V6	n.a*
V7	n.a*
*n.a.= not available. Different letters indicate significant difference among the experimental variants.	

The germination capacity of sorrel seeds was analysed for only five experimental variants (V1 to V5). Due to the massive microbiological contamination in the other two experimental variants, V6 and V7, the seed germination capacity could not be determined.

Comparing the collected data with those from the control, the best GC% was obtained when sorrel was germinated on perlite. No significant differences were seen between the control (V1) and V2 and V3 experimental variants. However, lowest values regarding GC% were registered in V5 variant, were sorrel was seeded in a substrate mixture of perlite 75% and organic banana peels 25%.

These results are similar to those obtained by Fani et al. (2012), testing the germination capacity of sheep sorrel (*Rumex acetosella* L.) in Petri dishes on soaked filter paper with solutions of different concentrations of gibberellic acid at 25/15°C (day/night) after four weeks.

2. Results on the number of leaves

The effect of substrate cultivation on the growth of sorrel sprouts in terms of the number of leaves is presented in Table 3.

Table 3. The number of leaves

Experimental variants	No. of leaves ± SD
V1	1.67±0.36
V2	1.47±0.53
V3	1.40±0.52
V4	1.57±0.60
V5	n.a*
V6	n.a*
V7	n.a*
*n.a.= not available	

Although the germination percentage results were recorded for V1 to V5 variants, the number of leaves indicator was evaluated only for the V1-V4 variants, as in the case of V5 variant the sprouts showed reduced growth. Moreover, the presence of banana peels in the substrate, negatively influence microplants growth, most probably due to the high microbial contamination of this intensive nourishing non-aseptic substrate.

Although some variations within the number of leaves were seen among the analysed experimental variants, statistically, there are no significant differences.

3. Results on dry matter content

Was performed for four experimental variants (Figure 1).

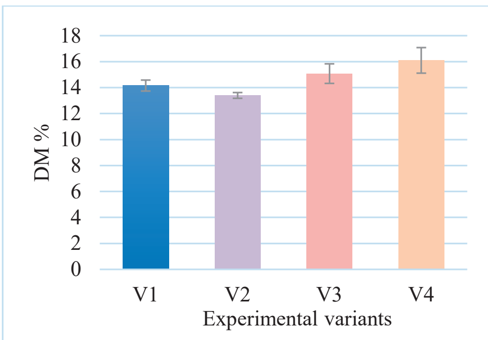

Figure 1. Dry matter content

Among these, the highest value was registered by the experimental variant V4 (16.10%), having as solid substrate perlite 75% and agar 25%. These results were significantly different from the control variant V1 (14.16%), were the solid substrate was based on perlite 100%. On the other hand, the lowest value was registered by the V2 (13.40%) variant, having as substrate two solid components, perlite 75% and agar 25%.

4. Results on the photosynthetic pigments

The effect of cultivation substrate on the accumulation of photosynthetic pigments of sorrel sprouts are presented in Figures 2, 3, and 4. The sorrel sprouts accumulated the highest photosynthetic pigments in the case of control variant with a value of 13.41 mg/g FW Chlorophyll a, while V4 having with 1.04 mg/g FW less (Figure 2).

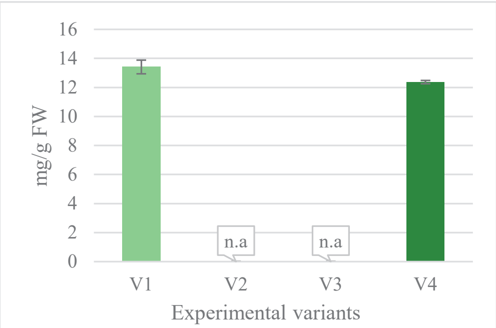

Figure 2. Total content of photosynthetic pigments - Clorophyll a

Comparative to the control, for V4 variant Chlorophyll b accumulation was with 1.03 mg/g FW less than of 11.82 mg/g FW in V1(Figure 3).

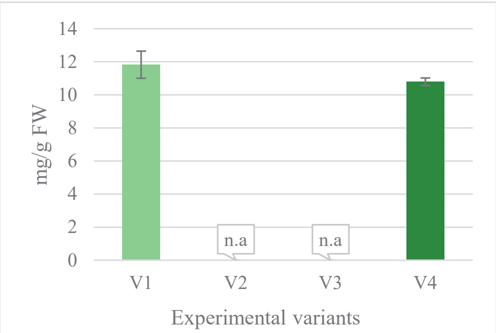

Figure 3. Total content of photosynthetic pigments - Clorophyll b

With regard to the total chlorophyll content, can be observed also an increase with 2.07 mg/g FW for control variant then V4 (23.16 mg/g FW) (Figure 4).

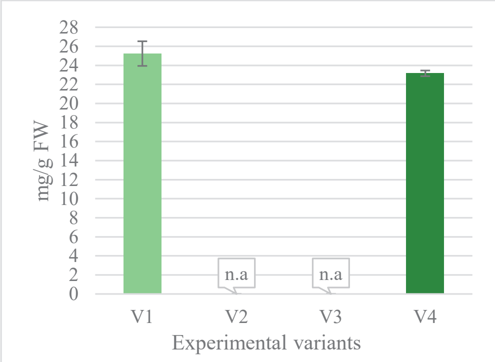

Figure 4. Total content of photosynthetic pigments

Our results are similar to those obtained by Kowitcharoen et al. (2021), who analyzed the total chlorophyll content in selected culinary microgreens that belong to *Brassicaceae, Fabaceae, Pedaliaceae, Polygonaceae Convolvulaceae* and *Malvaceae* families. The result registered for *Fagopyrum esculentum* Moench was of 37.85 mg/100 g, for *Pisum sativum* L. of 12.35 mg/100 g and for *Lens culinaris* Medicus of 112.62 mg/100 g.

5. Results on the micro and macronutrients

The micronutrients Mn, Cu, and Zn were determined and expressed as mg/Kg FW, while the macronutrients Na, Mg, P, K, Ca as mg/ 100 g FW.

The determination of Na content was performed for V1-V4 experimental variants. Among these, the highest value of the Na was registered by the experimental variant V2 (121.45 mg/100 g FW) compared to the control variant V1(113.80 mg/100 g FW). On the other hand, the lowest value was registered by the V4 variant, having 55.26 mg/100 g FW. The results are presented in Figure 5.

The determination of Mg showed that the highest value was registered by the experimental variant V2 with 41.34 mg/100 g FW comparative to the control variant V1 having 36.83 mg/100 g FW. On the other hand, the lowest value was registered by the V3 variant (33.33 mg/100 g FW). The results are presented in Figure 6.

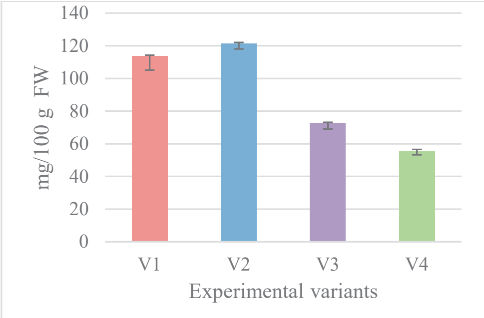

Figure 5. Sodium content in sorrel sprouts

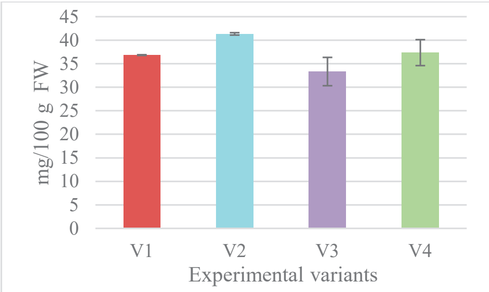

Figure 6. Magnesium content in sorrel sprouts

The determination of total phosphorus content was performed and the highest value was registered by the control experimental variant V1 (100.28 mg/100 g FW). Between V2-V4 variants a value of 94.287 mg/100 g FW was registered in the case of V4. On the other hand, the lowest value was registered by the V3 having as a nutrient substrate a solid component - perlite (50%) and agar (50%) with a value of 82.87 mg/100 g FW. For P the control variant has the highest values, so we can say that adding the agar to perlite lowers the P accumulation in sorrel, the best cultivation variant being 100% perlite. The results are presented in Figure 7.

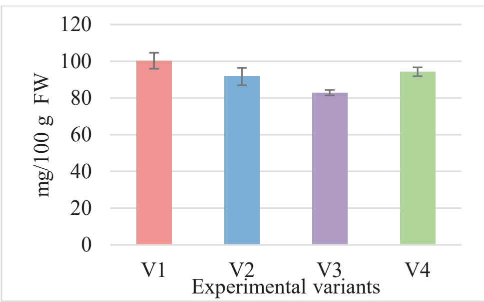

Figure 7. Phosphorus content in sorrel sprouts

The determination of total potassium content showed the highest value in V2, having 158.22 mg/100 g FW, while the control variant V1 123.80 mg/100 g FW. At the opposite pole, the lowest value was registered by the V4 with 143.78 mg/100 g FW. The results are presented in Figure 8.

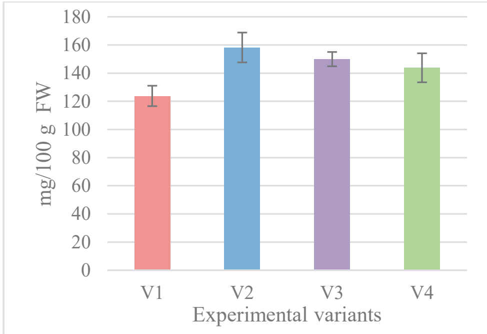

Figure 8. Potassium content in sorrel sprouts

The highest value of the Ca was registered by the experimental variant V4 (45.58 mg/100 g FW), variant having as a nutrient substrate a solid components perlite (25%) and agar (75%), compared to the control variant V1 (25.03 mg/100 g FW). On the other hand, the lowest value was registered by the V3 (33.82 mg/100 g FW) having as a nutrient substrate a solid components perlite (50%) and agar (50%). The results are presented in Figure 9.
For all the experimental variants, the content of calcium and potassium was higher than that of the control variant.

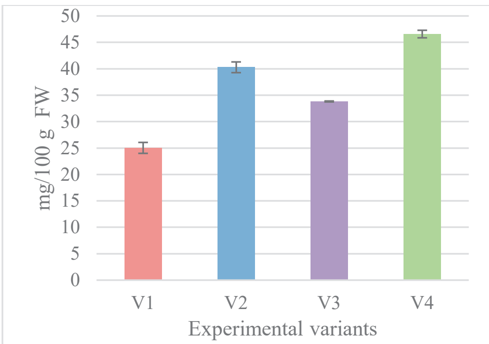

Figure 9. Calcium content in sorrel sprouts

Also, manganese content was performed for all experimental variants. Among these, the highest value of the manganese was registered in the case of V2 having a value of 17.95 mg/kg FW, comparative to the control variant V1 having 14.49 mg/kg FW. The lowest value was registered in the case of V3 having 14.41 mg/kg FW. The results are presented in Figure 10.

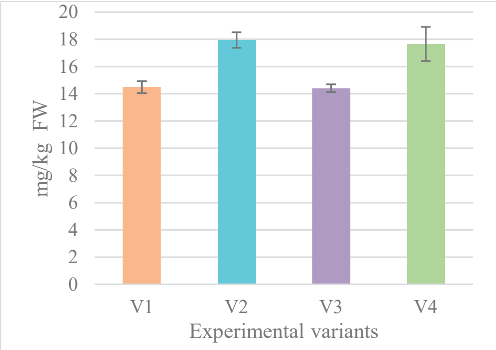

Figure 10. Manganese content in sorrel sprouts

Within micro nutrients, also copper content was quantified. Among all variants, the highest value of the copper was registered in experimental variant V4, recording 3.12 mg/kg FW, comparative to the control variant V1 having 0.61 mg/100 g FW. On the other hand, the lowest value was registered by the V2 having 0.5 mg/kg FW.

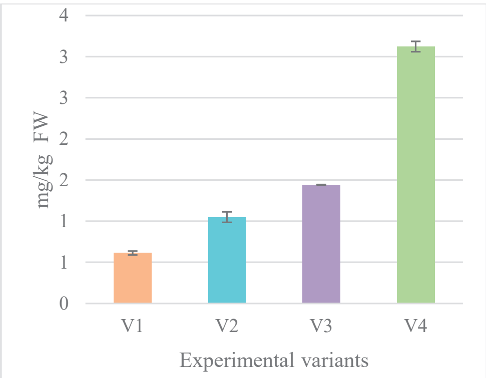

Figure 11. Copper content in sorrel sprouts

Continuing on the quantification of micro nutrients, zinc content was evaluated for all experimental variants. Among these, the highest value of the zinc was registered by the experimental variant V4, having as a substrate two solid components (perlite 50% and organic banana peels 50%) with a value of 7.82 mg/kg FW, greater than the control variant V1 having 6.74 mg/kg FW. Between variants, the lowest value was registered by the V2 having 6.82 mg/kg FW.

For all the experimental variants, the content of zinc and copper was higher than that of the control variant.

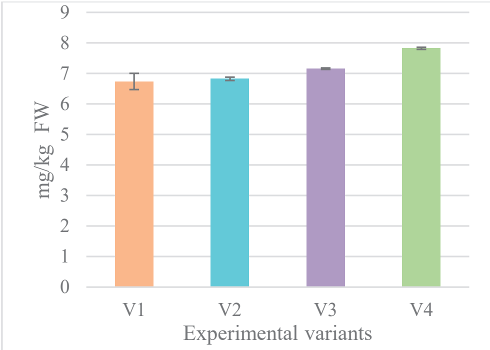

Figure 12. Zinc content sorrel sprouts

We can compare the results obtained for the qualitative analysis of sorrel sprouts regarding the content of macro and micronutrients with those obtained by Idris et al. (2011), who analysed dry leaves of mature plants of *Rumex acetosa* L., as follows: Na (28.61±0.48 mg/100 g DM), Mg (73.56±0.02 mg/100 g DM), P (7.73±0.07 mg/100g DM), K (2132.85±3.52 mg/100 g DM), Ca (53.25±0.05 mg/100 g DM), Mn (13.59±0.04 mg/100 g DM), Cu (0.85±0.04 mg/100 g DM), and Zn (2.66±0.01 mg/100 g DM).

According to these results, the recorded values demonstrate that the V2 variant with perlite 75% and agar 25%, had the highest content of macronutrients such as: Na (121.45 ± 3.42 mg/100 g FW), Mg (41.34 ±0.25 mg/100 g FW), K (158.22±10.59 mg/100 g FW), Ca (40.28 ± 1.02 mg/100 g FW) and Mn micronutrient (17.95 ±0.57 mg/kg FW).

In the V4 variant (perlite 25% and agar 75%) sorrel sprouts accumulated the highest Ca (46.58±0.714 mg/100 g FW), but for P content (94.29 ± 2.41 mg/100 g FW) the value is lower than the V1 control variant, but higher than V2 and V3. For micronutrients we can highlight the V4 results obtained in sorrel sprouts for Cu (3.12±0.06 mg/kg FW) and Zn (7.82± 0.04 mg/kg FW).

CONCLUSIONS

Comparing different cultivation substrates reveals that banana peels, in a non-aseptic system, is not suitable for sprouts (microplants) production. This fact banana peels are strongly related to the massive microbial contamination of V5, V6, V7 experimental variants.

The determination of total dry matter content of sorrel sprouts (microplants) indicates that V4 variant is optimal for this parameter.

The experimental variant V2 composed of perlite 75% and agar 25% registered significant values regarding the number of germinated seeds and the content of macro and micronutrients as Na, Mg, K and Mn.

Sorrel grown on V_4 variant based on perlite 25% and agar 75%, presented a high content of chlorophyll and macro and micronutrients as Ca, P, Cu and Zn.

Adding agar to the perlite cultivation substrate, lead to a decrease accumulation of P and assimilatory pigments content in sorrel sprouts (microplants).

It is essential to highlight the fact that the results indicate the influence of substrate cultivation on the biochemical content of sorrel sprouts (microplants). Considering these aspects, the preliminary results offer a perspective of sorrel sprout (microplants) production, thereby using the cultivation substrate associated with the accumulation of the health-promoting compound.

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RESEARCH ON THE SPECIES OF HEXAPODES EXISTING IN POTATO CROPS

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Abstract

To collect the material, were used Barber soil traps of the wet type, in which a 20% sodium chloride (NaCl) solution was placed. There were used 3 variants, each with 6 repetitions, as follows:V1, in which treatments were applied against pests, with products approved for organic agriculture; V2, in which treatments were applied against pathogens and pests in conventional agriculture; V3, in which no pest control treatment was applied. Collections of the captured material were made during the months of June, July and the first decade of August. The insect species collected belong to the following orders: Coleoptera, Hymenoptera, Diptera, Homoptera, Heteroptera, etc.

Key words: *Barber traps, variants, treatments, species, Coleoptera.*

INTRODUCTION

Insects, also called hexapods, constitute a class (according to some authors a super-class) of invertebrate animals. More than 1 million species are known. Entomologists believe that there are actually 3-4 million species in nature (Lonsdale, & Locke, 2018).

Insects live everywhere: in forests, in steppes, in rivers, lakes, in arid deserts and air - on the tops of the highest mountains. Many of them (flies, cockroaches, etc.) live near the human dwelling, and that is why they are called synanthropic insects.

The most varied in size and colour are the insects of tropical regions. Insects have a body from 0.2 mm (trichogrammes) to 33 cm (tropical phasmodes). They represent, according to estimates, 80% of animal species and are present in all climates and all environments. (Bitsch & Bitsch, 2000).

Biological control is one of the methods of protecting agricultural crops and involves the conscious intervention of specialists by introducing entomopathogenic or entomophagous organisms into agrobiocenoses, to regulate the density of pest populations when they exceed the PED.

Although introduced quite late as a cultivated plant in our country, the potato has a rich spectrum of pests, which can reduce the production of tubers/ha by 25-40%, sometimes they can even compromise it if the control measures are not applied according to the recommended technologies .

MATERIALS AND METHODS

The research was carried out in 2022 in a potato crop in the Rădăuți area, Marginea-Suceava commune, at the Varieties Testing Center, under the State Institute for Varieties Testing and Registration, Bucharest.

3 variants were used, as follows:
- V1, in which treatments against pests were applied, with products approved in organic agriculture;
- V2, in which treatments were applied against pathogens and pests in conventional agriculture;
- V3, in which no treatment against pests was applied.

The taxonomic study, distribution and ecological requirements were carried out on material collected from the field with the help of Barber traps. Different species of hexapods are captured by this method.

With the help of Barber traps, individuals of different species can be continuously collected, regardless of the biotope. After collecting and identifying the species, it is possible to assess the specific composition of the biocenosis, the seasonal variation and their cenotic preferences. Barber traps are 500 ml plastic boxes that are buried at ground level. The pits are made with the help of a small pickaxe or pickaxe. The boxes were buried carefully, so that the edge of the trap was at ground level, and the insects could easily enter. When the trap is installed for a longer time, a protective cover will be used to allow the access of insects and to prevent rainwater from entering the trap (Buburuz et al., 2013).The presence of the liquid excludes cannibalism between individuals captured in the trap and reduces the probability of their escape. The fixing liquid must have good preservative qualities to prevent maceration of the collected individuals. A 20% sodium chloride (NaCl) solution was used as a fixing liquid. (Tălmaciu et al., 2020).

Figure 1. Aspects from the field of experience

The number of traps that must be placed in a culture is a minimum of 6 and a maximum of 12, because with 5 traps the dominant, subdominant, recessive and subrecessive species are captured, and by using a number greater than 12 traps, the percentage ratios do not change significantly .

By placing 10-12 traps, all categories of species can be collected to establish dominance in a biotope, because in the case of a temperate climate we have two groups, one of species with large numbers (dominant, constant) and one of species with small numbers, sporadic. Therefore, the dominant species will always be collected. Through a small number of collections, only the abundant species (eudominant and dominant) will be captured. Through a larger number of samples, both the number of individuals and the collected species will be closer to the real number of herds in nature (Manole et al., 2009).

The contents of each box were placed on a cheesecloth sieve to separate the insects from the fixative. The gauze with each individual sample was placed in labeled bags. The label contains the following information: resident, culture, collection date and trap number. To preserve the elasticity of the insects and to anesthetize the live ones, acetic ether was used. After each collection, the trap was reinserted into the soil and the fixative fluid replaced. The collected material was brought to the laboratory, and the insects were determined and inventoried.

Collections of the captured material were made during the months of June, July and the first decade of August.

RESULTS AND DISCUSSIONS

In the year 2022, at the potato crop in the Rădăuți area, Marginea -Suceava commune, at the Variety Testing Center, subordinate to the State Institute for Testing and Registration of Varieties, at the V1 variant, in which treatments against pests were applied, with approved products in ecological agriculture, 431 hexapod specimens included in 5 orders were identified.

The product used in ecological pest control was Laser 240 SC with a dose of 100 ml/ha. Laser 240 SC has the efficacy of chemically synthesized insecticide (it has similar efficacy to pyrethroids and superior to organophosphorus and other chemically synthesized insecticides), but it is harmless to the environment and humans as it is a biological product (fermentation product of a soil bacterium: *Saccharopolyspora spinosae*). It belongs to a new family of biological insecticides: Naturalyte (comprises insecticides derived from metabolites of living organisms). Laser 240 SC is applied by spraying at warning, during the vegetation period. It has a residual effect of more than 3 weeks, depending on the climatic conditions. It is not washed off by rain two hours after application. The 433 specimens of hexapods were recorded, distributed as follows: *Coleoptera*, with 251 specimens, followed by the orders *Diptera* - 9 specimens, *Heteroptera* - 29 specimens, *Homoptera* - 52 specimens, *Hymenoptera*- 90 specimens and *Orthoptera* - 2 specimens (Table1, Figure 2).

Table 1. The hexapods collected in the V1

Ordinul	Specia	Total
Coleoptera	*Harpalus pubescens* Müller	53
	Anthicus antherinus L.	43
	Phyllotreta atra Fabricius	25
	Phyllotreta vittata Fabricius	21
	Silpha carinata Herbst	25
	Leptinotarsa decemlineata Say	14
	Longitarsus absinthii Kutschera	10
	Longitarsus luridus Scopoli	10
	Coccinella septempunctata L.	9
	Silpha obscura L.	8
	Aphthona euphorbiae Schrank	6
	Pleurophorus caesus Panzer	5
	Amara aenea De Geer	3
	Harpalus calceatus Duftschmid	3
	Longitarsus tabidus Fabricius	3
	Staphylinus caesareus Cederhjelm	3
	Harpalus tardus Panzer	2
	Hippodamia variegata Goeze	2
	Amara similata Gyllenhal	1
	Brachinus crepitans L.	1
	Harpalus aeneus Fabricius	1
	Harpalus azureus Fabricius	1
	Longitarsus ballotae Marsham	1
	Opatrum sabulosum L.	1
Diptera	*Anthomyiidae*	5
	Chloropidae	3
	Stratiomyidae	1
Heteroptera	*Pyrrhocoris apterus* L.	24
	Miridae	5
Homoptera	*Cicadellidae*	52
Hymenoptera	*Formicidae*	63
	Braconidae	13
	Ichneumonidae	12
	Apidae	2

In the V2 variant, 330 hexapod specimens included in 4 orders were identified. The order with the most specimens collected was *Coleoptera* (245 specimens), followed by *Homoptera* (colony); *Hymenoptera* (68 specimens); *Heteroptera* (9 specimens); *Diptera* (8 specimens) (Table 2, Figure 2).

The chemical products used in pest control were - Karate Zeon (50 g/l lambda-cyhalothrin) - 200 ml/ha, Mospilan 20 SG (acetamiprid 200 g/kg) - 100 g/ha, Coragen (Chlorantraniliprole 200 g/l) - 50 ml/ha, Faster 10 CE (Cypermethrin 100 g/l) - 200 ml/ha.

Table 2. The hexapods collected in the V2

Ordinul	Specia	Total
Coleoptera	*Harpalus pubescens* Müller	72
	Anthicus antherinus L.	38
	Coccinella septempunctata L.	31
	Aphthona euphorbiae Schrank	16
	Leptinotarsa decemlineata Say	12
	Silpha carinata Herbst	12
	Harpalus azureus Fabricius	10
	Phyllotreta atra Fabricius	9
	Harpalus calceatus Duft.	8
	Longitarsus tabidus Fabricius	7
	Harpalus aeneus Fabricius	6
	Opatrum sabulosum L.	6
	Amara aenea De Geer	5
	Athous mutilatus Rosenhauer	4
	Pseudophonus rufipes De Geer	3
	Dermestes bicolor Fabricius	2
	Longitarsus ballotae Marsham	2
	Pleurophorus caesus Panzer	2
Diptera	*Anthomyiidae*	8
Heteroptera	*Miridae*	6
	Dolycoris baccarum L.	2
	Eurydema oleracea L.	1
Hymenoptera	*Formicidae*	58
	Ichneumonidae	6
	Chalcididae	3
	Tiphiidae	1

In the V3 variant, in which no pest treatment was applied, 481 hexapod specimens were identified in 6 orders. The 481 specimens were recorded, distributed as follows: *Coleoptera*, with 255 specimens, followed by the orders *Diptera*, *Heteroptera*, *Homoptera*, *Hymenoptera* and *Orthoptera* (Table 3, Figure 2).

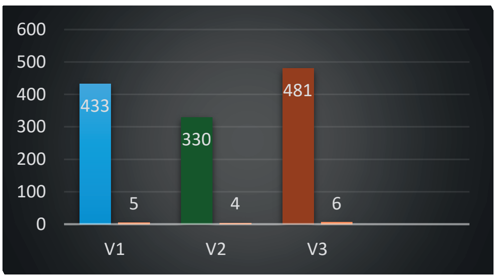

Figure 2. The situation of the collections on the three experimental variants

Table 3. The hexapods collected in the V3

Ordinul	Specia	**Total**
Coleoptera	*Leptinotarsa decemlineata* Say	106
	Harpalus pubescens Müller	41
	Anthicus antherinus L.	22
	Aphthona euphorbiae Schr.	20
	Coccinella septempunctata L.	20
	Drasterius fenestratus Küst	6
	Longitarsus tabidus Fabricius	6
	Pseudophonus rufipes De Geer	5
	Silpha carinata Herbst	4
	Staphylinus caesareus Cederh.	4
	Amara aenea De Geer	3
	Athous mutilatus Rosenhauer	3
	Phyllotreta atra Fabricius	3
	Formicomus pedestris Rossi	2
	Harpalus aeneus Fabricius	2
	Longitarsus ballotae Marsham	2
	Melanotus rufipes Herbst	2
	Adrastus limbatus Fabricius	1
	Harpalus calceatus Duftschmid	1
	Harpalus griseus Panzer	1
	Opatrum sabulosum L.	1
Diptera	*Anthomyiidae*	3
	Chloropidae	2
Heteroptera	*Miridae*	10
Homoptera	*Cicadellidae*	40
Hymenoptera	*Formicidae*	colony
	Torymidae	4
	Chalcididae	2
	Ichneumonidae	2
	Apidae	1
Orthoptera	*Gryllus campestris* L.	1

CONCLUSIONS

Potato crops are attacked by numerous pests and diseases, so there is a great potential to cause small to large yield losses in farmers' fields.

The application of chemical control must be done with great care to avoid pollution and effect on beneficial insects that would otherwise cause ecological imbalance. Again, most farm chemicals are toxic to farmers and should be applied with maximum protection.

In the V1 variant, in which treatments against pests were applied, with products approved in organic agriculture, 433 hexapod specimens were identified in 6 orders.

In the V2 variant, in which treatments were applied against pathogens and pests in conventional agriculture, 330 hexapod specimens were identified in 6 orders.

In the V3 variant, in which no pest treatment was applied, 481 hexapod specimens were identified in 6 orders.

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RESEARCH ON THE NUTRITIONAL VALUE, BIOACTIVE COMPOUNDS CONTENT AND ANTIOXIDANT CAPACITY OF SPIRULINA

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Abstract

Spirulina is a blue-green microalga, rich in bioactive compounds and nutrients. The high nutritional value and therapeutic potential of Spirulina have increased its global importance and the demand to make healthy food products fortified with this microalgae.The aim of this work was to determine the nutritional value, bioactive compounds and antioxidant capacity of Spirulina. The analyzed Spirulina samples stand out for their protein content (62.49-66.17%), total fiber (5.92-8.86%), total ash (6.22 -10.47%), vitamins (vitamin C: 55.45-82.73 mg/100 g; vitamin B1: 0.627-1.08 mg/100 g; vitamin B2: 2.12-3.84 mg/100 g; vitamin B3 (Niacin): 8.21- 12.83 mg/100 g; vitamin B5: 2.15-3.05 mg/ 100 g; vitamin B6: 0.653-0.875 mg/100 g; vitamin B9: 0.327-0.588 mg/100 g; vitamin B12: 0.105-0.127 mg/100 g), total polyphenols 154.50 -256.26 mg GAE/100 g), mineral elements (Na:85.77-120.45 mg/100 g; K:1385.37-1678.72 mg/1100 g; Ca: 850-1185.57 mg/100 g; Mg: 205.34-248.93 mg/100 g; Fe: 21.32-46.58 mg/100 g; Zn: 1.23-1.85 mg/100 g). Due to its high antioxidant content, Spirulina has antioxidant capacity (5.42-9.12 micromol Trolox/g). Due to its complex biochemical composition, Spirulina powder has antioxidant and therapeutic potential and can also be used for food fortification.

***Key words**: Spirulina, microalgae, nutrients, bioactive compounds, antioxidant capacity.*

INTRODUCTION

The exponential growth of the human population, climate change, water scarcity and the decrease of agricultural land constitute the global problems of society and a challenge for the production of food for the next generations. Given the exponential growth of the population and considering that several million tons of food are lost each year at various stages of the food chain, including production, post-harvest, processing and distribution, in the year 2050 will be necessary an increase of 50-60% of food production to feed everyone (Pereira et al., 2022). Microalgae have several attractive features for sustainable production at large scale, such as high biomass yields per unit area and the ability to be cultivated on non-arable land using non-potable water, or even salt water (Torres-Tiji et al., 2020).

Microalgae can be used as alternative foods with high nutritional value (Prihanto et al., 2022). *Spirulina* is a green-blue microalga, rich in bioactive compounds and nutrients (Shaban et al., 2017). *Spirulina* has been used both as a food and as a supplement (Moejes and Moejes, 2017).

Spirulina is produced in 23 countries, but the largest amounts of biomass are produced in Norway, France and Ireland (Araújo et al., 2021). *Spirulina* is of real interest for the food industry, due to its high content in proteins, minerals, vitamins, antioxidant carotenoid pigments and fatty acids and its high antioxidant capacity: proteins (35.4-70 g/ 100 g), fats (4-16g/100 g), carbohydrates (14-19 g/100 g), crude fiber (3-7g/100 g), mineral elements (Potassium: 2.0-2.6 g/100 g; Sodium: 1.5-2.2 g/100 g ; Phosphorus: 1.3-2.2 g/100 g; Iron: 273.2-787.0 mg/kg; Magnesium: 330 mg/100 g; Calcium: 120-900 mg/100 g), water-soluble vitamins (C: 115.03 mg/100 g; B12: 5.7-38.5 μg/100 g; B2: 3.0-4.6 mg/100 g; B6: 0.5-0.8 mg/100 g; B3 13-15 mg /100 g; B9: 0.05-9.92 mg/100 g); carotenoids (0.3-2.6 g/100 g), total phenolic compounds (0.20-1.73 g/100 g), polysaccharides (0.2-12.5 g/

100 g) (Ragaza et al., 2020; Rahim et al., 2021; Bensehaila et al., 2015).

The aim of this work was to determine the nutritional value, bioactive compounds and antioxidant capacity of *Spirulina.*

Also, *Spirulina* stands out for its fatty acid content. Mahardika et al. (2022), reported in the case of *Spirulina*, the following profile of fatty acids: 33.78% saturated fatty acids (such as docosanoic acid, octadecanoic acid, hexadecanoic acid and hexanedioic acid), 35.82% unsaturated fatty acids (fatty acids monounsaturated, diunsaturated fatty acids, triunsaturated fatty acids). The same authors showed in the research undertaken, that in the case of Spirulina, palmitic acid (hexadecanoic acid) has the highest content (25.21%).

Due to the complex biochemical composition, *Spirulina* and food products fortified with *Spirulina* have strong therapeutic effects in many conditions caused by oxidative damage such as neurodegenerative diseases, tumors, inflammation, immunosenescence, toxicosis, various internal organ damage, cardiovascular diseases, obesity, diabetes, etc. indicating enormous application potential in medicine and health (Han et al., 2021; Carrizzo et al., 2019; Huang et al., 2018; Neyrinck, 2017).

The aim of this work was to determine the nutritional value, bioactive compounds and antioxidant capacity of *Spirulina*.

MATERIALS AND METHODS

Materials

Spirulina samples were purchased from pharmacies (they are imported samples).

Figure 1 shows three *Spirulina* samples taken in the study.

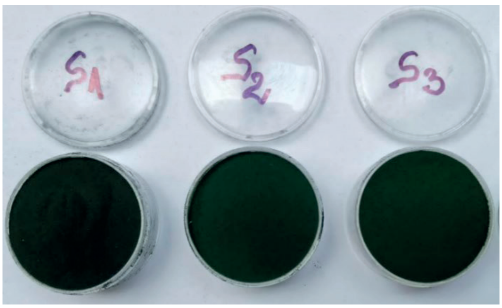

Figure 1. S*pirulina* powder samples

Methods

Statistical analysis

The *Spirulina* samples were analyzed in triplicate, and for each analytical parameter studied, the average value and standard deviation were reported.

Sensory analysis

Sensory analysis (appearance, taste, smell) was performed using the descriptive method.

Instrumental analysis of color (L*, a* and b*) was performed with CM-5 colorimeter (Konica Minolta, Japan), using SpectraMagic NX software.

Physico-chemical analysis

Moisture was determined by Halogen Moisture Analyzer HE53 (Mettler Toledo).

Chemical indicators have determined using AOAC Methods: 9 79.09 (protein content), 963.15 (fat content), 923.03 (ash content) and AOAC 985.29 (total dietary fiber). Total carbohydrate content (%) was estimated by subtracting water, fat, protein and ash content from 100%. The energy value calculation (kcal/100 g and kJ/100 g) was carried out according to the provisions of Commission Regulation no. 1169/2011 (European Commission, 2011).

Determination of iron, copper, manganese and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, model NexION300Q, Perkin Elmer) after dry digestion of the samples. The determination of sodium, potassium, calcium and magnesium was carried out by High-Resolution Continuum Source Atomic Absorption Spectrometry (Analytik Jena, model contrAA 700 - High-Resolution Continuum Source Atomic Absorption Spectrometer), flame technique, after dry digestion of the samples.

Bioactive compounds content

Total polyphenol content was performed by Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications, using UV-VIS Jasco V 550 spectrophotometer, at wavelength λ = 755 nm. The quantification of the total polyphenol content was based on the calibration curve of gallic acid (0-0.20 mg/mL).

The determination of the content in C and B vitamins was carried out by liquid chromatography (Accela chromatograph, Thermo Scientific) coupled with high-resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) (Asănică et al., 2019).

Antioxidant capacity

Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), using UV-VIS Jasco V 550 spectrophotometer, at wavelenght λ = 517 nm and calibration curve of Trolox (0-0.4375 mmol/L).

Microbiological analysis

The microbiological indicators were determined using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 method (*Enterobacteriaceae*), SR ISO 16649-2:2007 (*Escherichia coli*), SR EN ISO 6579-1:2017 (*Salmonella*), ISO 21807:2004 (Water activity). Water activity was determined using Aquaspector AQS 31 equipment.

RESULTS AND DISCUSSIONS

Sensory analysis

The sensory analysis revealed that *Spirulina* is presented in the form of a dark green powder and has a specific taste and smell.

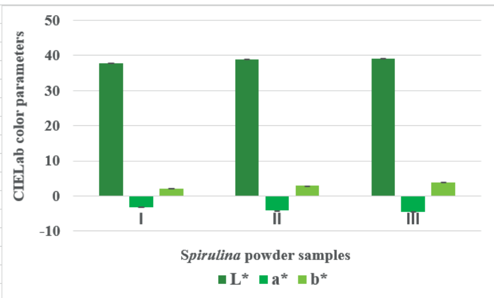

Figure 2. Colour parameters of *Spirulina* powder

Following the instrumental analysis of the color (Figure 2), it was found that the green powders obtained from cauliflower leaves had negative values of the a* parameter (-4.46...-3.14), positive values of the b* parameter (2.20... .3.86) and low luminance values (37.83...39.10).

The luminance values (L*) recorded in the case of the *Spirulina* powders in this study are higher than that reported by Demarco et al. (2022), in the case of *Spirulina* powder obtained through various dehydration processes (air drying, cast-tape drying, freeze-drying, vacuum cast-tape drying) from raw biomass of *Spirulina*: 13.09-20.30. The values of parameter a* (red-green color) and parameter b* fall within the ranges specified by Demarco et al. (2022), for these parameters, in the case of *Spirulina* powder (a*: -13.11...-3.76; b*: 1.64...10.88).

Physico-chemical analysis

The physico-chemical indicators of the *Spirulina* powder are presented in Table 1.

The *Spirulina* powders stand out for their content in protein (62.49-66.17%), ash (6.22-10.47%), carbohydrates (7.94-9.25%) and total fiber (5.92-8.86%).

Table 1. The physico-chemical indicators of *Spirulina* powder

Physico-chemical indicators	*Spirulina* powders		
	Sample I	Sample II	Sample III
Moisture (%)	7.13±0.18	5.90±0.15	8.73±0.22
Ash (%)	6.22±0.09	6.34±0.09	10.47±0.16
Protein (%)	64.18±0.96	66.17±0.99	62.49±0.94
Fat (%)	4.53±0.06	5.78±0.08	3.14±0.04
Total fiber (%)	8.86±0.16	7.87±0.15	5.92±0.11
Carbohydrates (%)	9.08±0.06	7.94±0.05	9.25±0.06
Energy value (kcal/100g)	316	333	303
Energy value (kJ/100g)	1333	1402	1282

The moisture content of *Spirulina* powder in this study is in the range reported by other authors: 5.81-10.1 % (Koli et al., 2022; Raczyk et al., 2022; Ali, 2022; Matos et al., 2016). It is important to ensure a maximum humidity of 10.5%, in the case of *Spirulina* powder, to ensure the microbiological stability of this microalgae.

Ash is an important chemical indicator that, in the case of the studied *Spirulina* powders, varied in the range of 6.22-10.47% (the minimum value was recorded in the case of Sample I, and the maximum value in the case of Sample III).

The ash content of these *Spirulina* samples is higher compared to those reported by Raczyk et al. (2022) (Ash content = 5.93%) and Saharan and Jood (2017) (Ash content = 3.5%). Sample III of *Spirulina* powder has the highest ash content, compared to the other two analyzed samples and, at the same time, higher than those reported by Koli et al. (2022) (Ash content = 8.34%) and Tańska et al. (2017) (Ash content = 7.90%).

The protein content of *Spirulina* powder taken in the study is higher compared to those reported by Ali (2022) (Protein content = 56.2%), Lafarga et al. (2020) (Protein content = 57.5 %), comparable to that reported by Koli et al. (2022) (Protein content = 65.71%), but lower than those reported by Raczyk et al. (2022) (Protein content = 71.34%), Saharan and Jood (2017) (Protein content = 71.90%). *Spirulina* is the richest natural source of digestible protein that provides all essential amino acids to the human body (Guil-Guerrero et al., 2004).

High digestibility of *Spirulina* is due to the fact that its cell wall is made up of 86% from digestible polysaccharides (Li and Qi, 1997). Also, it is worth emphasizing that the total nucleic acid content of *Spirulina* is in the range of 4.2-6% d.m. and does not present any risk of increasing plasma uric acid when consuming up to 10 g/day (Soni et al., 2020). Capelli and Cysewski (2010) mention that *Spirulina* contains protein as high as 670% compared to tofu. In many countries in Africa, *Spirulina* is still used as human food because it is a major source of protein, and thus, it is collected from natural water, dried and consumed. Today, *Spirulina* is used in many countries as a disease prevention strategy to maintain health (Koli et al., 2022). The consumption of *Spirulina* lead to a series of beneficial health effects, having immunomodulatory, antioxidant, anticancer, antiviral and antibacterial properties. At the same time, the consumption of *Spirulina* have positive effects against malnutrition, anemia, hyperlipidemia, diabetes, obesity, inflammatory allergic reactions, toxicity induced by heavy metals (Wu et al., 2016).

The lipid content of the analyzed *Spirulina* powders varied between 3.14-5.78%, being higher than those reported by other authors: Raczyk et al., 2022 (Fat content = 0.36%) and Saharan and Jood, 2017 (Fat content = 1.27%). The main fatty acids contained in *Spirulina* are gamma-linolenic acid, linoleic acid and palmitic acid (Esquivel-Hernández et al., 2016). Among these fatty acids, gamma-linolenic acid is known to be a functional compound of *Spirulina* lipids, along with carotenoids, tocopherols and sterols, because there are not many food sources that contain a significant content of gamma-linolenic acid (Esquivel-Hernández et al., 2016; 2017; Gutiérrez-Salmeán et al., 2015; Ku et al., 2013). At the same time, gamma-linolenic acid is the precursor of prostaglandins, leukotrienes and thromboxanes that are mediators in inflammatory processes, in immune processes, participating in the prevention of several chronic inflammatory diseases and cancers (Das, 2004; Gutiérrez-Salmeán et al., 2015; Sergeant et al., 2016)

The total fiber content of the *Spirulina* powders taken in the study varied in the range: 5.92-8.86 % (the minimum value was recorded in the case of Sample III, and the maximum value in the case of Sample I). Raczyk et al. (2022) reported in the case of *Spirulina* powder a total fiber content of 8.45%. Also, in the case of *Spirulina* powder, Morsy et al. (2014) report a fiber content of 7.93%, and Saharan and Jood (2017), reported a fiber content of 14.98%. Fiber consumption has beneficial effects on the human body (lowering cholesterol levels, helping to control sugar levels and maintaining gut health), it is beneficial to enrich our diet with fiber (Ötles, and Ozgoz, 2014; Fuller et al., 2016).

The carbohydrate content of the *Spirulina* powders analyzed in this study varied between 7.94-9.25 (the minimum value was recorded in the case of Sample II, and the maximum value in the case of Sample III). The carbohydrate content of these *Spirulina* samples is higher than that reported by Raczyk et al. (2022) (Carbohydrates content = 6.83 %), but lower than those reported by Koli et al. (2022) (Carbohydrates content = 21.87%) and Saharan and Sudesh (2017) (Carbohydrates content = 13.63%). *Chlorella vulgaris* and *Spirulina* produce sulfated polysaccharides that are considered nutraceuticals, being recommended in the prevention and/or treatment of cancer (Carbone et al., 2021; Kiran et al., 2021).

The chemical composition of *Spirulina* powder can vary due to environmental conditions, water composition, climate and salinity (Ali, 2022).

The energy value of the *Spirulina* powders studied is comparable to that reported by Raczyk et al. (2022), in the case of this microalgae (333.4 kcal/100 g, respectively 1395.0 kJ/100 g).

The *Spirulina* powders are notable for their content in mineral elements (Na, K, Ca, Mg, Fe, Zn, Cu and Mn), which is presented in Figures 3, 4 and 5.

Among the mineral elements, in the case of *Spirulina* powders, potassium has the highest content, in the range of 1385.37-1678.72 mg/100 g (the minimum value was recorded in the case of sample I, and the maximum value in sample III).

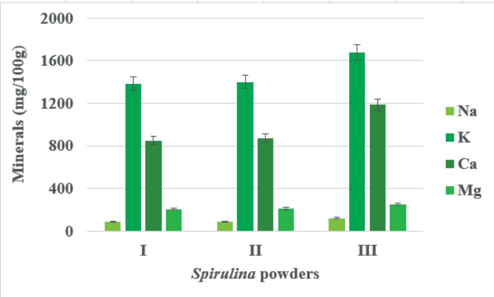

Figure 3. Mineral content (Na, K, Ca, and Mg) of the *Spirulina* powder

The potassium content of *Spirulina* powders (sample I and sample II) is comparable to that reported by Lafarga et al. (2020) in the case of this microalga. Sample III has a higher potassium content than that reported by these authors.

The sodium content of *Spirulina* powder analyzed in the study undertaken (85.77-120.45 mg/100g) is significantly lower than that reported by Lafarga et al. (2020), in the case of this microalga.

Spirulina is a microalga recognized for its high level of calcium, which can be used as an alternative source of calcium. The highest calcium content is recorded in the case of *Spirulina* cultivated with sea water (Ekantari et al., 2017). *Spirulina* powders analyzed in our study had a calcium content in the range of 850-1185.57 mg/100 g, the highest value being registered in the case of sample III. The calcium content of *Spirulina* samples from our study is 7.88-11 times more than that reported by Koli et al., 2022 (107.83 mg/100 g) and, respectively, 1.37-1.91 times more compared to that reported by Saharan and Jood, 2017 (620.80 mg/100 g). At the same time, the calcium content of these samples is comparable to that reported by Sharoba (2014), in the case of *Spirulina* powder: 922,278 mg/100 g. Ekantari et al. (2017) conducted a study in which they demonstrated that the bioavailability of calcium from *Spirulina* is higher than that from milk and calcium carbonate. As well, Capelli and Cysewski (2010) mention the fact that *Spirulina* contains calcium as high as 180% compared to milk.

The magnesium content of *Spirulina* powder in our study (205.34-248.93 mg/100 g) is higher than that reported by Lafarga et al. (2020), in the case of the same microalgae: 195 mg/100 g.

Spirulina powder is an important source of iron. The iron content of the studied *Spirulina* (Figure 4) was in the range of 21.32-46.58 mg/100 g, the highest content being recorded in the case of sample III.

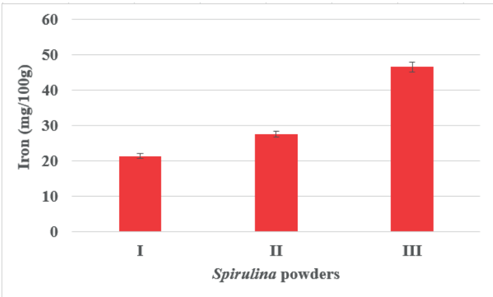

Figure 4. Iron content of the *Spirulina* powder

The iron content of samples I and II is comparable to that reported in the case of *Spirulina* powder by Lafarga et al., 2020 (28.5 mg/100 g) and Koli et al., 2020 (26.59 mg/ 100 g), respectively. The iron content of sample III is 1.63 times and 1.75 times higher, respectively, than that reported by the authors mentioned above, in the case of *Spirulina* powder. Capelli and Cysewski (2010) mention that *Spirulina* contains iron as high as 5100% compared to spinach. *Spirulina* is an important source of iron containing 20 times more iron than a gram of wheat. Iron is a mineral that is mainly present in foods of animal origin such as meat and fish (Balasubramani et al., 2016). Consuming *Spirulina* is very beneficial especially for athletes, vegetarians, pregnant women and teenagers (Soni et al., 2017). Selmi et al. (2011) showed that supplementing the

diet with *Spirulina* increases hemoglobin levels and improves the immune system in the elderly.
The zinc content of the analyzed *Spirulina* powder (1.23-1.85 mg/100 g) is lower than that reported by Lafarga et al., 2020 (2.0 mg/100 g). The zinc content of sample III is comparable to that reported by Koli et al., 2022 (1.87 mg/100 g).

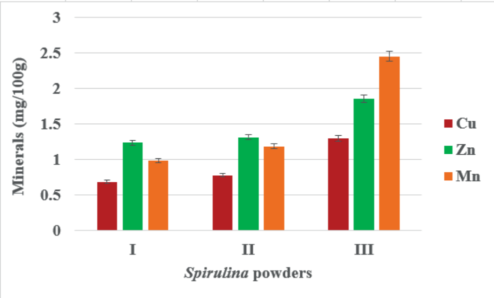

Figure 5. Mineral content (Zn, Cu and Mn) of the *Spirulina* powder

The copper content of the *Spirulina* powder samples taken in the study is comparable to that reported by Sharoba (2014) (1.2154 mg/100 g), but 4.73-8.9 times lower compared to that reported by Lafarga et al., 2020 (6.1 mg/100 g). The manganese content of the three *Spirulina* powder samples varied in the range of 0.98-2.45 mg/100 g, being comparable to that reported by Lafarga et al. (2020) (1.9 mg/100 g)

Bioactive compounds content

The *Spirulina* powders stand out for their content in bioactive compounds: total polyphenols, vitamin C, B group vitamins, β-Carotene and lutein. The content in bioactive compounds of the *Spirulina* powders is presented in Table 2.

Table 2. Bioactive compounds content of the *Spirulina* powder

Bioactive compounds	*Spirulina* powders		
	Sample I	Sample II	Sample III
Total polyphenols (mg GAE/g)	154.50±3.86	223.74±5.59	256.26±6.41
Vitamin C (mg/100g)	55.45±1.66	61.84±1.85	82.73±2.48
Vitamin B1(mg/100g)	0.627±0.019	0.745±0.022	1.08±0.032
Vitamin B2 (mg/100g)	2.12±0.06	2.23±0.07	3.84±0.12
Vitamin B3 (Niacin) (mg/100g)	8.21±0.25	9.12±0.27	12.83±0.38
Vitamin B5 (mg/100g)	2.15±0.06	2.42±0.07	3.05±0.09
Vitamin B6 (mg/100g)	0.653±0.020	0.687±0.021	0.875±0.026
Vitamin B9 (mg/100g)	0.327±0.010	0.346±0.010	0.588±0.017
Vitamin B12 (mg/100g)	0.105±0.003	0.112±0.003	0.127±0.004
β-carotene (mg/100g)	185.21±4.26	201.64±4.64	325,45±7.48
Lutein (mg/100g)	10.67±0.25	12.32±0.28	15.85±0.36

The total polyphenol content of *Spirulina* powders varied in the range of 154.50 – 256.26 mg GAE/100g, being higher than that reported in 2017 by Kuatrakul et al., were in the case of *Spirulina* powder obtained by hot air dehydration of fresh *Spirulina*: 99.76 mg GAE/100 g d.m. Also, the total polyphenol content of sample III is comparable to that reported by Kuatrakul et al., 2017, in the case of *Spirulina* powder obtained by microwave dehydration of fresh *Spirulina*: 273.26 mg GAE/100 g d.m. The content of total polyphenols of *Spirulina* powder analyzed in the study is lower than that reported by other authors in the case of *Spirulina*: 408 mg GAE/100 g (Rodríguez De Marco et al., 2014), respectively, 484.2 mg mg GAE/100 g (drying under vacuum at 60°C) and 607.6 mg GAE/100 g (drying at 55°C) (Larrosa et al., 2016). Rose et al. (2023) mention the fact that the differences in total polyphenol content, in the case of *Spirulina* powder samples procured from various sources, can be explained by the different stages of maturity and harvesting, respectively processing (such as grinding and drying conditions) of the *Spirulina* used as raw material.
The vitamin C content of the *Spirulina* samples analyzed varied between 55.45-82.73 mg/100 g, and it was lower compared to that reported by Rahim et al. (2021) for this microalgae: 115.03 mg/100 g. The highest vitamin C content was recorded in sample III (87.23 mg/100 g). The vitamin B1 content of the *Spirulina* powder taken in the study varied between 0.627-1.08 mg/100 g, being lower than that reported by Lafarga et al. (2020): 2.4 mg/100 g. The vitamin B2 content from the *Spirulina* samples is higher compared to the vitamin B1 content and varied between 2.12-3.84 mg/100 g. The vitamin B2 content of sample III is comparable to that reported by Lafarga et al., 2020 (3.7 mg/100 g), but lower compared to that reported by Liestianty et al. (2019), in the case of *Spirulina* powder: 5.5 mg/100 g. The vitamin B3 content of the Spirulina III sample is similar to that reported by Lafarga et al., 2020 (12.8 mg/100 g), but lower by 14.67% compared to that reported by Liestianty et al. (2019): 15 mg/100 g. The vitamin B5 content of *Spirulina* powders varied between 2.15-3.05 mg/100 g, being about 10-

15 times higher than that reported by Liestianty et al. (2019): 0.2 mg/100 g.
The vitamin B6 content of *Spirulina* powder in this study (0.653-0.875 mg/100 g) is higher than that reported by Lafarga et al. 2020 (0.4 mg/100 g), but it is comparable to that reported by Liestianty et al. (2019), in the case of this microalgae: 0.8 mg/100 g.
The vitamin B9 content of the *Spirulina* samples taken in the study was in the range of 0.327-0.588 mg/100 g, being 4.6-8.3 times higher than that reported by Liestianty et al., 2019 (0.071 mg/100 g), but lower than that reported by Rahim et al. (2021), for *Spirulina*: 0.799 mg/100 g. The vitamin B12 content of the analyzed *Spirulina* samples was within a narrow range (0.105-0.127 mg/100 g), being lower than that reported by Sharoba et al., 2014 (0.175 mg/100g) and respectively Liestianty et al., 2019: 0.360 mg/100g. Soni et al. (2017) mention that dehydration processes of *Spirulina* biomass influence the retention of nutrients and bioactive compounds in *Spirulina* powder.
Thus, the different values recorded for the water-soluble vitamin content of this microalga could be explained.
Spirulina powder is an important source of β-carotene. In the case of *Spirulina* powder from this study, the β-carotene content varied between 185.21-325.45 mg/100g, being higher than that reported by Ljubic et al., 2018, (174 mg/100g d.m.), respectively, that reported by Hynstova et al., 2018 (86.85-103.71mg/100g), in the case of this microalgae. Sharoba (2014) reported for β-carotene content a value of 252.7 mg/100g, comparable to that obtained in this study.
Other carotenoids identified in *Spirulina*, but in lower concentrations, include canthaxanthin and lutein (Hynstova et al., 2018). In the case of the analyzed *Spirulina* samples, the lutein content was in the range of 10.67-15.85 mg/100g, being comparable to the minimum value of the range reported by Hynstova et al., 2018, in the case of *Spirulina*: 11.78-103.09 mg/100 g.
Cerón-García et al. (2018) mention that natural carotenoids are preferred compared to synthetic ones, as they are a mixture of trans and cis isomers. Synthetic carotenoids are usually all-trans isomers.

Antioxidant capacity
Due to the high content in antioxidants, *Spirulina* powder taken in the study, shows antioxidant activity. Their antioxidant activity varied between 5.42-9.12 µmol TE/g (the minimum value was recorded in sample I, and the maximum value in sample III) (Figure 6).

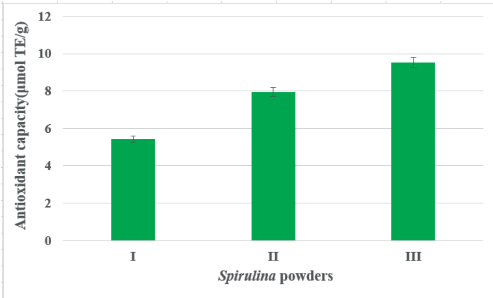

Figure 6. Antioxidant capacity of the *Spirulina* powder

For the *Spirulina* powders between the total polyphenol content and antioxidant capacity it is a linear correlation ($y = 0.0397x - 0.7674$; $R^2 = 0.995$).

Microbiological analysis
The microbiological indicators of *Spirulina* powders are presented in Table 3.

Table 3. Microbiological analysis of the *Spirulina* powder

Microbiological indicators	*Spirulina* powders		
	Sample I	Sample II	Sample III
Yeasts and molds (CFU/g)	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10
Escherichia coli (CFU/g)	< 10	< 10	< 10
Salmonella (in 25 g)	absent	absent	absent
Water activity	0.285	0.263	0.301

The results of the microbiological analysis confirmed the fact that these powders fall within the provisions of the legislation in force. At the same time, it is worth noting that the *Spirulina* powders have low values for water activity (0.285-0.301), which ensures their stability from a qualitative point of view.

CONCLUSIONS

Spirulina is a valuable biological resource, due to its high content in nutrients and bioactive compounds, which give its antioxidant capacity. The *Spirulina* studied stands out for its content in protein (62.49-66.17%), ash

(6.22-10.47%), carbohydrates (7.94-9.25%) and total fiber (5.92-8.86%). It is also worth noting the high mineral content of this microalgae (potassium 1385.37-1678.72 mg/100; calcium: 850-1185.57 mg/100 g; magnesium: 205.34-248.93 mg/100 g; sodium: 85.77-120.45 mg/100 g; iron: 21.32-46.58 mg/100 g; zinc: 1.23-1.85 mg/100 g; manganese: 0.98-2.45 mg/100 g; copper: 0.68-1.29 mg/100 g).

Also, *Spirulina* powder is a source of bioactive compounds: total polyphenols (154.50 – 256.26 mg GAE/100g), vitamin C (55.45-87.23 mg/100g), vitamin B1 (0.627-1.08 mg/100 g) vitamin B2-3 (100 g) mg/100 g), vitamin B3 (8.21-12.83 mg/100 g), B5 (2.15-3.05 mg/ 100 g), β-Carotene (185.21-325.47 mg/100 g) , lutein (10.67-15.85 mg/100 g).

Due to the high antioxidant content, *Spirulina* powder showed antioxidant capacity (5.42-9.12 μmol TE/g).

The microbiological analyzes carried out have highlighted that *Spirulina* powders comply with the legislation in force and are safe for human consumption.

Being an important source of nutrients and bioactive compounds *Spirulina* powder can be used in the fortification process of food products in order to increase their nutritional value and potential.

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RESEARCH ON THE CAPITALIZATION OF THE NUTRITIONAL AND BIOACTIVE POTENTIAL OF CAULIFLOWER LEAVES IN THE CIRCULAR ECONOMY CONTEXT

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Abstract

Globally, it is estimated that a third of all food produced is lost. This is driven by many different factors, but the lack of consumer awareness of the nutritional value and antioxidant potential of certain parts of the food, namely peels, seeds, stems is certainly among them. The objective of this work was to capitalize on the nutritional and bioactive potential of cauliflower waste (leaves), in order to obtain a functional ingredient with high nutritional value and antioxidant potential. This functional ingredient is presented in the form of a homogeneous, microbiologically stable powder and stands out for its content in protein (28.72-29.95%), ash (10.39-11.23%), glucosinolates (240.23-259.65 mmol/g s.u.) total fiber (25.11-30.45%), vitamin C (76.50-89.44 mg/100g), β-carotene (39.45 -47.21 mg/100g), total phenolic compounds (5.75-6.12 mg GAE/g s.u.), mineral elements (Fe: 40.75-51.15 mg/100g; K: 3865.52-4178.21 mg/100g; Ca: 772.23-812.21 mg/100g: Mg: 120.23-145.85 mg/100g; Zn: 5.45-7.78 mg/100g). Due to the high antioxidant content, the functional ingredient obtained from cauliflower leaves has an antioxidant capacity: 13.36 - 14.2 µmol TE/g. Due to its complex biochemical composition and antioxidant capacity, this functional ingredient from cauliflower leaves can be used in the food fortification process.

Key words*: cauliflower, leaves, functional ingredient, antioxidant capacity.*

INTRODUCTION

According to estimations of the UN organization, the world population will reach 9.8 billion by 2050, almost 20% higher than today (U.N.D.E. and S.A., 2019). The exponential growth of the human population, climate change, water scarcity and the decrease of agricultural areas are the global problems of society and a challenge for the production of food for the next generations. Globally, it is estimated that a third of all food produced is lost. This is due to several factors, but the lack of consumer awareness of the nutritional value and antioxidant potential of certain food parts, such as peels, seeds, stems, leaves, is certainly among them (Pereira et al. , 2022).

Plant by-products represent more than a third of all food waste (Bharat Helkar and Sahoo, 2016). In the case of Brassica species, a high percentage of waste results: 45-60% in the case of cauliflower and 60-75% in the case of broccoli (Khedkar et al., 2017; Petkowicz and Williams, 2020; Castelãdo-Baptista et al., 2021). Research undertaken internationally (Drabińska et al., 2021) highlights the fact that cauliflower waste (stems and leaves) are sources of nutrients and bioactive compounds, which can be exploited. Thus, from the results of the research undertaken by these authors, it is highlighted that cauliflower leaves and stems have a content in glucosinolates, phenolic compounds, mineral elements, total amino acids, fibers, higher than cauliflower (inflorescence). At the same time, cauliflower leaves have a higher antioxidant capacity than the inflorescence (leaves: 150 µmol Trolox Equivalents/g d.m.; inflorescence: 133.75 µmol Trolox Equivalents/g d.m.), due to the higher content in bioactive compounds (Drabińska et al., 2021).

International epidemiological studies have highlighted the anticancer effects of isothiocyanates, which are biologically active

molecules derived from the enzymatic degradation of glucosinolates (Arumugam and Razis, 2018).
At the same time, cauliflower leaves are a rich source of β carotene, iron and calcium and therefore can be used in the composition of value-added products to treat anemia and nutritional deficiencies (Shivani et al., 2018). Wani and Kaul (2011) showed that dehydrated dried cauliflower leaves are rich in nutrients and are a good source of β carotene (43.11 mg/100 g) iron (60.38 mg/100 g), copper (1.55 mg /100 g), manganese (5.86 mg/100 g), zinc (5.10 mg /100 g). Carotenoids, especially lutein and β-carotene, which are most abundant in *Brassicaceae*, contribute to the reduction of oxidative stress and related disorders such as cancer, diabetes, and cardiovascular diseases (Gul et al., 2015; Jahangir et al., 2009).
It is important to utilize cauliflower waste in functional ingredients with high nutritional value and antioxidant capacity. For this purpose their transformation into powders is of real interest (Salehi, 2020; Santos et al., 2022).
This paper presents the sensory, nutritional, microbiological characteristics, the content in bioactive compounds and the antioxidant capacity of the functional ingredient (powder), obtained by using cauliflower leaves.

MATERIALS AND METHODS

Samples

Cauliflower leaves were procured from Romanian farmers. The experiments undertaken to obtain the functional ingredient (powder) from cauliflower leaves were carried out in the Vegetable-Fruit Processing Pilot Experiment Station, within IBA Bucharest. The technological flow for obtaining this functional ingredient (powder) includes the following operations: sorting, washing, boiling in water at 98-100°C for 2-3 minutes, cutting, dehydration, grinding and packaging.
Dehydration of the plant material was carried out at 50°C, in an electric dryer with forced convection, for 8-9 hours, up to a maximum humidity of 8%. Grinding of the dried plant material was carried out using a Retsch mill, at a temperature of 20-22°C, to ensure the preservation of the nutritional characteristics of the functional ingredient. The functional ingredient obtained from cauliflower leaves was packed in hermetically sealed glass containers and protected with aluminum foil and kept in dry and cool spaces (at a maximum temperature of 20°C), till the sensory, biochemical and microbiological analyses were performed. The Figure 1 show cauliflower leaf powder (three samples).

Figure 1. Cauliflower leaf powder from three different sources (farmers)

Methods

Statistical analysis

The cauliflower leaf powder samples were analyzed in triplicate, and the results obtained for each analytical parameter studied were reported as the average value and standard deviation.

Sensory analysis

Sensory analysis (appearance, taste, smell) was performed using the descriptive method (the method is based on sensory characteristics).

Instrumental analysis of color (L*, a* and b*) was performed with CM-5 colorimeter (Konica Minolta, Japan), using SpectraMagic NX software.

Physico-chemical analysis

Moisture was determined by Halogen Moisture Analyzer HE53 (Mettler Toledo). Chemical indicators were determined using AOAC Methods: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content) and AOAC 985.29 (total dietary fiber). Total carbohydrate content was estimated by subtracting water, fat, protein and ash content from 100%. The energy value calculation (kcal/100 g and kJ/100 g) was carried out according to the provisions of Commission Regulation no. 1169/2011 (European Commission, 2011).
Determination of iron, copper, manganese and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, model

NexION300Q, Perkin Elmer) after dry digestion of the samples. The determination of sodium, potassium, calcium and magnesium was carried out by High-Resolution Continuum Source Atomic Absorption Spectrometry (Analytik Jena, model contrAA 700 - High-Resolution Continuum Source Atomic Absorption Spectrometer), flame technique, after dry digestion of the samples.

Bioactive compounds content

Total polyphenol content was performed by Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications, using UV-VIS Jasco V 550 spectrophotometer, at wavelength λ = 755 nm. The quantification of the total polyphenol content was based on the calibration curve of gallic acid (0-0.20 mg/mL). The determination of β-carotene and lutein content was carried out by chromatographic method (High Performance Liquid Chromatograph with Diode array detector) (Catană et al., 2020). The determination of vitamin C content was carried out by liquid chromatography (Accela chromatograph, Thermo Scientific) coupled with high-resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) (Catană et al., 2017). The determination of glycosinolate content was carried out using a rapid spectrophotometric method (Mawlong et al., 2017).

Antioxidant capacity

Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), using UV-VIS Jasco V 550 spectrophotometer, at wavelenght λ = 517 nm and calibration curve of Trolox (0-0.4375 mmol/L).

Microbiological analysis

The microbiological indicators were determined using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 method (*Enterobacteriaceae*), ISO 21807:2004 (Water activity). Water activity was determined using Aquaspector AQS 31 equipment.

RESULTS AND DISCUSSIONS

Sensory analysis

Sensory analysis revealed that the functional ingredient obtained by recovery of cauliflower leaves looks like a green powder with a characteristic taste and smell for *Cruciferous* family.

Following the instrumental *analysis of the color* (Figure 2), it was found that the green powders obtained from cauliflower leaves had negative values of the a* parameter (-4.62...-4.55), positive values of the b* parameter (11.42.. .11.74) and low luminance values (48.32...48.55). The luminance values (L*) recorded in the case of the cauliflower leaf powders in this study are higher than the one reported by Drabiańska et al. (2021), in the case of the powder obtained from cauliflower leaves by lyophilization and grinding: L* = 34.24. Also, the value recorded for the a* parameter was higher than that reported by Drabiańska et al. (2021) (a* = -5.13), and the b* parameter registered a lower value than that reported by the same authors: b* = 13.36.

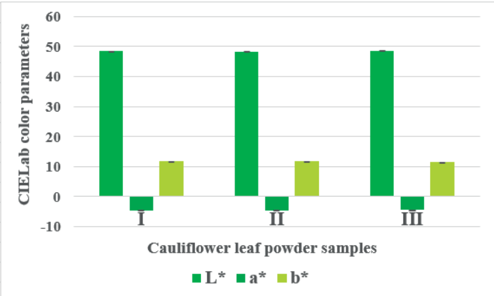

Figure 2. Colour parameters of the cauliflower leaf powder

Physico-chemical analysis

The physico-chemical indicators of the cauliflower leaf powder are presented in Table 1. The cauliflower leaf powders stand out for their protein content (28.72-29.95%), ash (10.39-11.23%), total sugars (17.85-21.05%) and total fiber (25.11-30.45%).

The protein content of cauliflower leaf powders obtained in this experimental study is higher compared to that reported by Chakraborty and Datta De (2016), 27.78 % and respectively by Perna et al. (2019), 21.16 % d.m., in the case of this functional ingredient.

Table 1. The physico-chemical indicators of the cauliflower leaf powder

Physico-chemical indicators	Cauliflower leaf powder		
	Sample I	Sample II	Sample III
Moisture (%)	7.30±0.18	7.45±0.19	7.12±0.18
Ash (%)	10.39±0.16	10.65±0.16	11.23±0.17
Protein (%)	28.72±0.43	29.08±0.44	29.95±0.45
Fat (%)	4.25±0.06	4.53±0.06	4.77±0.06
Carbohydrates (%)	49.34±0.32	48.29±0.31	46.93±0.30
Soluble sugars (%)	17.85±0.12	18.65±0.12	21.05±0.14
Total fiber (%)	30.45±0.56	29.03±0.54	25.11±0.46
Energy value (kcal/100g)	290	292	300
Energy value (kJ/100g)	1210	1222	1257

According to the provisions of Regulation (EC) No 1924/2006 of the European Parliament and of the Council, the cauliflower leaf powders are sources of proteins since at least 12% of their energy value is represented by proteins. Therefore, this functional ingredient can be used for fortification of food products in order to increase its protein content.

The ash content of cauliflower leaf powders obtained in this experimental study is higher compared to that reported by Drabiańska et al. (2021), in the case of fine powder obtained by freeze-dried and ground cauliflower leaves: 10.13 %. The high ash content of the functional ingredient obtained by utilizing cauliflower leaves actually highlights its high mineral element content.

It is worth noting the content in soluble sugars of the cauliflower leaf powder: 17.85-21.05%, respectively, 19.26% s.u.- 22.67% s.u. Collado-González et al. (2022) mention the fact that in the case of cauliflower leaves, the soluble sugars are represented by fructose, glucose, sucrose and inositol, their content being in the range: 15.96% s.u.- 44.58% s.u. The content of soluble sugars of the cauliflower leaf powders obtained in this study is located in the range mentioned above, reported by Collado-González et al. (2022).

The cauliflower leaf powder is an important source of fiber. The fiber content of the cauliflower leaf powders obtained in our research is lower than that reported by Ribeiro et al. (2015), in the case of cauliflower leaves flour 33.22%. It should be noted that the cauliflower leaf powders have a fiber content of 1.35-1.64 times higher than the fiber content of dried cauliflower, reported by Baloch et al. (2015): 18.59%.

Dietary fiber has a major impact on human health. Thus, they can have direct and indirect effects on the immune system when introduced into the diet. Before being fermented by microbes in the colon, dietary fiber can have a substantial impact on the gut by modulating intestinal barrier function and the immune response (Cai et al., 2020).

At the same time, Bhoite et al. (2021) mention that foods rich in protein and dietary fiber could improve the lipid profile in overweight or obese diabetic patients with dyslipidemia, reducing their risk of cardiovascular disease. These authors showed in a study carried out for 24 weeks, on a group of overweight/obese adults, the fact that, along with an adequate diet, the twice-daily administration of a nutritional supplement with high fiber and protein content, caused a significant increase in HDL-cholesterol levels and a modest decrease in LDL-cholesterol levels.

Considering the fact that the cauliflower leaf powders have a high protein and fiber content, their use for the fortification of food products (bakery, pastry, etc.) or for making a food supplement is of real interest.

The cauliflower leaf powders are notable for their content in mineral elements (Na, K, Ca, Mg, Fe, Zn, Cu and Mn), which is presented in Figures 3 and 4. Among the mineral elements, in the case of the cauliflower leaf powders, potassium has the highest content, in the range of 3865.52-4178.21 mg/100 g (the minimum value was recorded in the case of sample I, and the maximum value in sample III). The potassium content of the cauliflower leaf powders is higher than that reported by Saleh (2022) in the case of cauliflower wastes powder: 3462.69 mg/100 g.

Several international studies have shown that potassium is an important mineral for the proper function of the human body. An adequate diet with an adequate level of potassium is important for the prevention of cancer and cardiovascular diseases (Kardalas et al., 2018; Tabasum et al., 2014). The World Health Organization recommends that adults consume at least 3,500 mg per day from food.

At the same time, the cauliflower leaf powders stand out for their high calcium content (772.23-812.21 mg/100 g), and can be considered a source of calcium in the food fortification process.

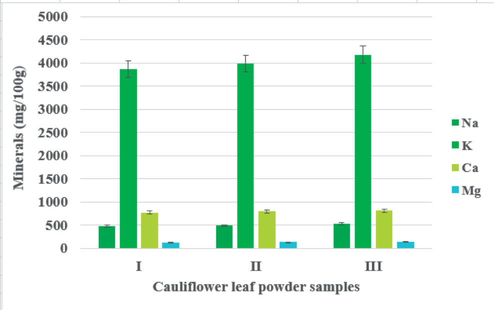

Figure 3. Mineral content (Na, K, Ca, and Mg) of the cauliflower leaf powder

The calcium content of the cauliflower leaf powders obtained in this study is higher than that reported by Saleh (2022) in the case of cauliflower waste powder: 750.40 mg/100 g. Also, it is worth noting that the cauliflower leaf powders have a calcium content about 2 times higher than that reported by Baloch et al. (2015), in the case of dried cauliflower: 395.03 mg/100 g. Calcium is one of the most abundant elements in the human body, which has a key role in skeletal mineralization, being necessary for normal bone growth, development and strength (Bootman et al., 2001). In addition, calcium plays a role in biological functions such as muscle contraction and nerve impulse transmission (Khundmiri et al., 2016).

The magnesium content of the cauliflower leaf powders varied between 120.23-145.85 mg/100 g, being higher than that reported by Saleh (2022) in the case of cauliflower wastes powder: 114.03 mg/100 g. At the same time, it is worth noting that the cauliflower leaf powders have higher magnesium content than that reported by Baloch et al. (2015), in the case of dried cauliflower: 117.87 mg/100 g.

Magnesium is one of the most important micronutrients, being essential for maintaining the normal function of cells and organs (Porri et al., 2021). At the same time, magnesium is important for maintaining muscle function and the nervous system, cardiac electrical properties and for supporting the immune system, as well as regulating glucose and insulin metabolism (Volpe, 2013).

The cauliflower leaf powders have an important sodium content, the highest value being registered in the case of sample III: 525.73 mg/100 g.

The cauliflower leaf powders can be considered sources of iron, as they recorded a high iron content, in the range of 40.75-51.15 mg/100 g (Figure 4).

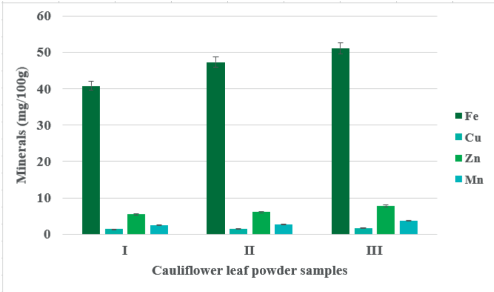

Figure 4. Mineral content (Fe, Zn, Cu and Mn) of the cauliflower leaf powder

According to the experimental data obtained, the iron content of the cauliflower leaf powders is higher than that reported by Saleh (2022) in the case of cauliflower waste powder: 37.85 mg/100 g. Iron has an essential role in cellular metabolism. The reduced form of iron is a cofactor in numerous redox reactions in the cell, intervening in many vital physiological functions. Most of the iron exists in the form bound to proteins, in erythrocytes in the form of hemoglobin and in storage proteins, such as ferritin, hemosiderin and myoglobin. Iron is also bound to non-heme proteins and enzymes involved in oxidation-reduction reactions and electron transfer (Salnikow, 2021). Iron is an important element in the treatment of nutritional deficiencies and iron deficiency anemia. Singh et al. (2019) studied the influence of some foods fortified with cauliflower leaf powder, on the nutritional status of rural school children from India. These fortified foods were administered to study participants (girls and boys aged 10-12 years) along with their daily diet for three months. At the end of the study, there was an increase in the height of the subjects (an average of 2.11 cm for boys and, respectively, an average of 1.26 cm for girls), an increase in weight (an average increase of 13.45% in the case of boys and 14.66% in the case of girls), increase in hemoglobin values (20.12%, in the case of boys of age 10 year+ and 12.83%, in the case of boys of age 11 year+; 8.33% in the case of girls of age 10 year+ and 13.75% of age

11 year+). The conclusion of the study was that the use of cauliflower leaf powder for the fortification of food products could improve the nutritional status of the population, controlling micronutrient deficiencies, especially iron deficiency anemia.

The zinc content of the cauliflower leaf powders recorded values in the range of 5.45-7.78 mg/100 g, lower than that reported by Saleh (2022), in the case of this functional ingredient: 23.45 mg/100 g. Zinc is an essential micronutrient for the health of the human body, intervening in the proper functioning of various cellular processes. In particular, zinc plays an important role in cellular antioxidant defense, the maintenance of DNA integrity, and the development and normal functioning of the immune system (Ho et al., 2022).

The manganese content of the cauliflower leaf powders was in the range of 2.52-3.63 mg/100 g, higher compared to that reported by Saleh (2022), in this functional ingredient: 0.14 mg/100 g.

The copper content of the cauliflower leaf powders recorded lower values, compared to the other elements identified and quantified in their case (1.39-1.67 mg/100 g).

Bioactive compounds content

Cauliflower leaf powders are notable for their content in bioactive compounds: total polyphenols (5.75-6.12 mg GAE/g d.u.), glucosinolates (240.23 - 259.65 mmol/g d.u.), vitamin C (76.50 - 89.44 mg/g s.u.), β-Carotene (39.45 - 47.21 mg/100 g), lutein (11.65 - 14.87 mg/100 g). The bioactive compound content of cauliflower leaf powders is shown in Table 2.

Table 2. Bioactive compounds content of the cauliflower leaf powder

Bioactive compounds	Cauliflower leaf powder		
	Sample I	Sample II	Sample III
Total polyphenols (mg GAE/g s.u.)	5.75±0.14	5.92±0.15	6.12±0.15
Glucosinolates (mmol/g s.u.)	240.23±5.77	252.12±6.05	259.65±6.23
Vitamin C (mg/100g)	76.50±2.29	80.23±2.41	89.44±2.68
β-Carotene (mg/100g)	39.45±0.91	42.49±0.98	47.21±1.09
Lutein (mg/100g)	11.65±0.27	13.24±0.30	14.87±0.34

The total polyphenol content of cauliflower leaf powders obtained in this experimental study is higher than that reported by Drabiańska et al. (2021), in the case of fine powder obtained from lyophilized and ground cauliflower leaves: 4.40 mg GAE/g.s.u.

Polyphenols are a very important class of phytochemical compounds and have several beneficial health effects due to their antioxidant and anti-inflammatory properties.

Epidemiological studies have shown a lower risk of chronic diseases (cardiovascular diseases, cancer, hypertension, diabetes) and neurodegenerative diseases, in the case of people who approach a diet rich in polyphenols (Voss et al., 2022).

The cauliflower leaf powders are important sources of glucosinolates, the content of these bioactive compounds being in the range of 240.23-259.65 mmol/g s.u. The content of these bioactive compounds is higher than that reported by Drabiańska et al. (2021), in the case of cauliflower leaf powder 225.2 mmol/g s.u. It is worth noting that the cauliflower leaf powders have a glycosinolate content of 2.55-2.76 times higher than the powder obtained by freeze-dried and ground of florets, which according to the research undertaken by Drabiańska et al. (2021), has a glycosinolate content of 94.02 mmol/g s.u. Glucosinolates are highly valuable bioactive compounds for health, providing protection against serious diseases such as colorectal cancer, prostate cancer, breast cancer, and myocardial infarction (Akram et al., 2021).

Also, the cauliflower leaf powders have a high content in vitamin C (76.50-89.44 mg/100 g), comparable to that of fresh cauliflower (florets). Vitamin C is an important antioxidant. Thus, vitamin C is involved in the primary prevention of coronary heart disease, stroke and cancer (Granger and Eck, 2018).

The cauliflower leaf powders are notable for their carotenoid content. Thus, the β-Carotene (39.45-47.21 mg/100 g) and lutein (11.65-14.87 mg/100 g) content of this functional ingredient is higher, compared to that reported for these bioactive compounds by Nartea et al. (2023), in the case of cauliflower leaf flour (β-Carotene: 35.73 mg/100 g; Lutein: 10.35 mg/100 g). Carotenoids are phytochemical compounds with important photoprotective and antioxidant properties. Dietary supplementation with carotenoids provides protection against diabetes, inflammatory diseases, and cancer (Swapni et al., 2021)

Antioxidant capacity

Due to the high antioxidant content, the cauliflower leaf powders show important antioxidant activity. Their antioxidant activity varied between 13.36 - 14.2 μmol TE/g (the minimum value was recorded in the case of sample I, and the maximum value in sample III) (Figure 5).

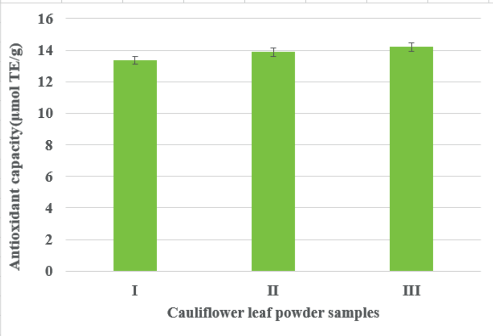

Figure 5. Antioxidant capacity of the of the cauliflower leaf powder

For the cauliflower leaf powders between the total polyphenol content and antioxidant capacity it is a linear correlation ($y = 2.2507x + 0.4665$; $R^2 = 0.9667$).

Microbiological analysis

The microbiological indicators of the cauliflower leaf powders are presented in Table 3.The results of the microbiological analysis confirmed the fact that these powders fall within the provisions of the legislation in force. At the same time, it should be noted that of the cauliflower leaf powders have low values for water activity (0.180-0.203), which ensures their stability from a qualitative point of view.

Table 3. Microbiological analysis of the cauliflower leaf powder

Microbiological indicators	Cauliflower leaf powder		
	Sample I	Sample II	Sample III
Yeasts and molds (CFU/g)	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10
Water activity	0.194	0.203	0.180

The results of the microbiological analysis highlight the fact that the cauliflower leaf powders can be used under food safety conditions, in the fortification of food products.

CONCLUSIONS

The research undertaken has highlighted the high nutritional and bioactive potential of cauliflower leaves and the fact that they should not be regarded as waste, but should be considered as sources of nutrients and bioactive compounds. The functional ingredients (powders) obtained by utilizing cauliflower leaves stand out for their protein content (28.72-29.95%), ash (10.39-11.23%), total sugars (17.85-21.05%) and total fiber (25.11-30.45%). It is also worth noting the high content of mineral elements (potassium 3865.52-4178.21 mg/100 g; calcium: 772.23-812.21 mg/100g; magnesium: 120.23- 145.85 mg/100g; sodium: 475.58-525.73 mg/100 g; iron: 40.75-51.15 mg/100 g; zinc: 5.45-7.78 mg/100 g; manganese: 2.52-3.63 mg/100 g; copper: 1.39-1.67 mg/100 g).

At the same time, these functional ingredients are sources of bioactive compounds: total polyphenols (5.75-6.12 mg GAE/g s.u.), glucosinolates (240.23 - 259.65 mmol/g s.u.), vitamin C (76.50 - 89.44 mg/100 g), β-Carotene (39.45 - 47.21 mg/100 g), lutein (11.65 - 14.87 mg/100 g).

The microbiological analyzes carried out showed that the cauliflower leaf powders are safe for human consumption.

Due to the high content in nutrients, bioactive compounds and antioxidant potential, the cauliflower leaf powders can be used in food safe conditions, in the process of fortification of food products (for example, bakery products, pastries), in order to increase the nutritional value and of their antioxidant capacity.

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IMPLEMENTATION OF GOOD NUTRITIONAL PRACTICES TO STIMULATE MEAT PRODUCTION IN SHEEP USING SAME HORTICULTURAL SPECIES

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Abstract

To reduce the pressure of sheep on plant resources, good nutritional practice models must provide new farming systems and feed management models, which reduce the pressure on grasslands by developing sheep farms integrated in the production of fodder in agricultural crops and on mowing pastures, well-managed grazing and improving the floristic composition of meadows and increasing their nutritional value, by administering phosphorus fertilizers for the development of nitrogen-fixing leguminous species or using organic fertilizers to increase the production of green mass. The good nutritional practices proposed for implementation contribute to the improvement of feed assimilation by sheep in different exploitation systems, because breastfeeding plays a fundamental role in the nutritional intake due to the intake of nutrients from the feed, milk production and weaning weight of the lambs.

Key words*: sheep, meat, nutrition, good practices.*

INTRODUCTION

Achieving large productions from sheep in efficient conditions is conditioned by several key points of the production system that must be solved by the management implemented at the farm level (Csizmadia et al., 2023; Nuthal, 2010):
- improving production systems by abandoning traditional systems and implementing modern exploitation technologies through nutrition control; (Heber et al., 2010);
- implementation according to the financial strength of the farm of the best management of the production system (Petroman et al., 2019);
- improving the livestock and specializing the production according to the needs of the market (Avramescu et al., 2013);
- stimulating the organization of farms through association for increasing the breeding material and the fattening of young sheep (Petroman et al., 2010; Ristea et al., 2018);
- supporting the milk and sheep meat product and stimulating consumption;
- marketing of production through distribution through the product chain (Adzig et al., 2018).

In climatic conditions of Romania, the exploitation of sheep for different productions, milk, meat or wool in different production systems, is done depending on the season, using the value of pastures during April-October months and during the stable period from warm season green table, administered mowing at the manger (Csizmadia et al., 2021). In winter, in the feed of sheep in different physiological states, canned fodder, hay, coarse and concentrated, but also other resources from the processing industries are used. The stable system of animal production presents a multitude of economic advantages over grazing methods because it is easier to control nutrition by balancing feed rations and improve feed conversion rates, resulting in:
- a higher consumption degree of fodder plants;
- a better balancing of rations;
- feeding possibilities with unique feed mixtures;
- better yields in using fodder;
- a better utilization of green fodder;
- quantitative and qualitative indicators of productions (Căpeț et al., 2021);
- constant productions (Petroman, 2007).

Exploitation on unmanaged pasture can produce pressure on meadows, due to overgrazing and their degradation, imbalances of environmental factors and requiring major investments for their regeneration:

- getting good gains in weight gain and large quantities of milk;
- scaling the production according to the possibilities of balancing rations and resources (Petroman, 2016);
- exploitation of plant resources in sheep feed without degrading environmental factors, through optimization the livestock;
- diversification of farm activities;
- limiting grain consumption through efficient use of green fodder (Vîrtosu, 2019);
- increasing the economic efficiency of sheep farming (Zoican, 2019).

MATERIALS AND METHODS

The efficient use of plant resources in sheep's feed depends on the management of the production system, their availability, quantity and quality in the stable operation, but also on the adaptability degree of sheep to provide the necessary by grazing on the meadows, with a certain value of the floristic composition, by plants maturity degree of and their suitability for grazing according to the category of sheep and the management methods of grazing.

Organic fertilization of meadows with manure, raking, leads to a change in their floristic composition and reseeding with valuable fodder plants and the establishment of artificial pastures with species of *Festuca pratensis*, *Trifolium repens* and *Lolium perenne*, increases their fodder value, if the grazing is well managed for a good use of plant resources.

The methods of efficient use of plant resources through grazing, allow the improvement of forage assimilation through the good practices of:

- continuous grazing, which involves the uninterrupted use of a grazing area by sheep of different categories, for a long period of time, the number of which can be adjusted to the growth of the vegetation of the pasture to meet the requirements of the sheep;
- rotational grazing, which involves grazing only in certain periods interspersed with breaks to allow the pasture to regenerate using:

• grazing on plots, in which the grazed area is adjusted according to the daily feed requirements of the sheep category;

• preferential grazing, in which milk lambs have access to a separate pasture area, of better quality;

• "leaders and followers" grazing, in which the pasture is grazed first by the group of sheep with higher requirements, leaders (lactating sheep) and then by the group of sheep with lower requirements, followers (barren sheep).

Based on research undertaken in professional sheep farms, specific methods were used in this scientific approach to analyze the efficiency of nutrition systems, depending on the production system (Gruia, 2006). The research aimed to find the most optimal solutions for managing nutritional resources and administration methods according to availability, season, quantities, and the possibilities of balancing them in rations through:

1. **Nutrition control according to the forecasted production in accordance with the biological potential of sheep**. For a good assimilation of the feed in the production system in the stable, under economical conditions, we propose the administration of mowed green feed, depending on the category and age, 1.1-2.2 kg/day for lambs, 3.1-4.0 kg/day in the youth over 6 months and 7.1-8.5 kg/day in the adult sheep because on the meadows in the farm area, the production of green mass in the ungrazed and repeatedly mowed meadows achieved 2.2 tons/hectare S.U. compared to those grazed with loads of 2 UVM (large cattle units) per hectare where 2.18 tons/hectare of SU were achieved and at 4 UVM/ha and above, thanks to overgrazing, 1.50 tons of SU/hectare were achieved, because sheep exert a negative impact on meadows where the nutritionally valuable species, *Trifolium repens* mixed with *Lolium perenne*, predominate. The controlled administration of the amount of feed, in the stable, presents economic advantages, which reflect on the achieved production indicators:

- increases the degree of consumption of mowed green fodder to 80.1-85.2% compared to 70.1-77.3% if this fodder is consumed by grazing;
- the spread of diseases is prevented;
- the entire production of green mass is efficiently utilized through less selective consumption.

For efficient meat production in fattening young, single feed rations should have the following percentage composition 67.00% fibrous forage (40% hay and 60% roughage), 18.00% silage, green mass, and 15.00% corn cobs. The deer will be supplemented with concentrated fodder and a protein-mineral-vitamin supplement to balance the ration and ensure the salt requirement through lumps of salt for licking. In order to reduce the pressure of the sheep through overgrazing, the load on the plant resources on the meadows will be reduced of sheep in the case of those meadows that have productions of green mass below 5 tons/hectare.

Good nutritional practices also contribute to the improvement of feed assimilation by sheep both in the barn and on the pasture. Because lactation plays a fundamental role in the nutritional intake of sheep: lactating sheep consume much more than other sheep. However, a highly variable correlation between feed intake and milk production was observed:

- the ability to assimilate food after calving does not automatically lead to an increase in milk production;
- the lactation period is the phase in which the sheep mobilize fat reserves to satisfy increased nutritional requirements.

The good nutritional practices should not provide for the estimation of feed assimilation capacity during the lactation period of the sheep, but should take into account through the nutrition systems:

- the physiological tendency to mobilize fat reserves in the first months of lactation;
- restoration of fat reserves later.

The best and safest method of controlling nutrition according to the forecasted production of Turkana sheep is the management of mowed green mass and well-managed grazing on rich permanent pastures, because the sheep can have access to a feed of superior quality in terms of nutritional principles, which can be easily assimilated.

2. **Good practices of using available resources based on a nutrition guide** (Neagu et al., 2002; Neagu et al., 2007). The best practices for the use of pasture resources, in the exploitation of sheep on pasture and a new exploitation system, through the administration of mowed fodder, which will reduce the pressure on the plant resource, are:

- the development of sheep farms integrated in the production of fodder in agricultural crops;
- the use of pastures for the production of fodder resources by mowing;
- well-managed grazing to maintain a balance regarding the productive capacity of the pasture and the load of sheep per hectare;
- transition to ecological agriculture;
- the cultivation of fodder plants on agricultural land and the management of mowed green mass;
- maximize profits from raising sheep in the open air and managing sufficient amounts of mown green mass according to planned production;
- improving the floristic composition of meadows by administering phosphorus fertilizers for the development of leguminous species that fix nitrogen;
- the use of organic fertilizers to increase the production of green mass of meadows.

These systems of best practices regarding the use of available resources cannot be effective without the development of integrated farming systems by combining:

- the production of crops to ensure the need for green mass, nutritional needs;
- the management of the exploitation of sheep in the open air, with the management of the green mass mowed in association with concentrates;
- sheep farming systems for the production of milk - meat through good nutritional practices, the culture of fodder plants can be integrated with the growth and exploitation of sheep, to create jobs throughout the year and additional income, the purpose being multiple;
- ensuring the additional and complementary relations of the professional sheep farm for the use of by-products of one component of the agricultural system as input for another;
- maintenance of soil fertility;
- obtaining a maximum profit per surface unit;
- improving ecological diversity;
- a high-performance management system in the management of available plant resources and of potential production is the nutritional balance with growth processes in lambs, youth, rams and ewes. No matter for what purpose this system is practiced, engineering genetics

correlates nutritional needs with the productivity of the natural environment.

Although the researches mostly address only the management of the farm resources, we believe that an improved management of the use of the available plant resources is required based on the best management practices that provide for:

- plotting and fencing pastures- for the efficient use of plant resources;
- the location of grazing areas near the farm;
- decontamination of grazing areas;
- avoiding contact with other animals.

Improved grazing and rotation management can reduce the area of pastures, integrated grazing and feeding systems, the use of manure to fertilize meadows enriches plant production, the palatability and nutritional quality of green mass, possibilities to cultivate pastures with other crops and hayfields can be converted into habitats naturally, contributing to the rehabilitation of the natural environment.

3. **Balancing rations according to economic efficiency** (Nicholson, 1994). Seasonal productivity of pastures can contribute to increasing the efficiency of fattening by balancing feed rations with concentrates according to production specialization and reducing the negative impact on the environment. The rations will be balanced in such a way as to ensure:

- grouped calvings in February a good correlation between the nutritional requirements on the milk production cycle and the feed produced naturally after the lambs are weaned at 42 days;
- staggered calvings through hormonal stimulation of estrus and feeding with estrogenic fodder that ensures the continuity of production, the alignment of production with natural processes and involves the implementation of an approachable farm nutritional management for any production system and type of exploitation;
- preserving the benefit of prolificacy and saving surplus lambs used for meat production by using milk substitutes in lamb rations. For one liter of sheep's milk substitute, 1000 milliliters of cow's milk, 40 grams of egg melange and 60 grams of milk powder with the chemical composition of:
- dry substance - 20.45%;
- crude protein - 14.80%;
- crude fat - 3.87%.

Administered in the feed of lambs regardless of the natural or artificial lactation system from day 8, the requirement being supplemented with combined feed (1.05 UN and 141.8 grams of digestible crude protein/kg (PBD) and the energy-protein ratio of 134.38 grams PBD/UN the following results were obtained:

- at 14 days, naturally breastfed lambs achieved an average weight of 10.90±0.60 kg;
- artificially breastfed lambs 11.10±0.33 kg average weight.

After weaning at 42 days, both categories of lambs were exploited in the barn, their rations were balanced according to performance, physiological and growth requirements, 1.02 UN, 115.50 grams PBD/gram and the energy-protein ratio of 113.23 PBD/UN and 200 grams of alfalfa hay.

During the fattening period from 42-70 days, the lambs that were fed naturally with mother's milk during the lactation period achieved average daily weight gains of 200.30-271.42 grams and those artificially breastfed 215.70-342.20 grams, contributing to the achievement of the best growth, and the combined feed administered in the feed from the age of 8 days.

Balancing fodder rations according to production category and age regardless of the stable or pasture management system, improving grazing management and pasture rotation:

- reduce the area of pastures;
- integrated grazing and feeding systems improve feed conversion indices and contributes to obtaining large daily increases in weight, over 300 grams;
- the use of manure enriches plant production;
- rotation, optimization of herds depending on the degree of supportability of the meadow and its use of unique forages improve the palatability and nutritional quality of the green mass.

RESULTS AND DISCUSSIONS

For a good consumption, without losses, measures are required to ensure a good feed assimilation through the implemented production system and obtaining productions:

-as close as possible to the genetic value of the herd;
-economical through the price of the resources used in the rations:
In this regard, for stable operation, we propose a set of good practices for quantitative forage with mowed green mass to avoid wastage by category of sheep:
- lambs according to weight category: 1.0-2.0 kg;
-youth over the age of 6 months 3.0-3.8 kg;
-adult sheep 6.5-8.0 kg.
The good practices of controlled quantitative administration of the mowed green mass, in the stable system, present the following economic advantages for professional farms, with implications on the indices through:
- stimulation of consumption, due to the freshness of the administered fodder;
- increasing the degree of assimilation through mowed management to over 85% compared to grazing which does not exceed 58.0-76.0%:
- sanitary-veterinary control of specific diseases, transmitted by: parasites, viruses, bacteria;
- efficient utilization of green mass resources;
- avoiding the selective consumption of fodder plants in the case of grazing.

These good feeding practices in sheep production in the stable system are more efficient because almost the entire amount is consumed due to the fact that needs can be correlated with expected productions, unlike unmanaged grazing where sheep are selective and prefer only certain varieties of plants depending on development the stage of consumes only certain parts of the vegetal plants carpet. Stimulantly feeding the lambs in the barn from 8 days of age with combined feed and increasing amounts of alfalfa hay from 200-500 grams contributes to:
- achieving good economic results, weights of 18.30-24.70 per 42 days;
- weights of 30-37.5 kg at 70 days;
- consumption of 250-550 grams of combined feed.

In uncontrolled grazing there is a waste of fodder resources, the sheep preferentially consume the plants from the floristic composition of the meadow only in proportion of 75-77%.

The production of milk in sheep exploited on pasture, increases per surface unit along with the growth rate of herds to the maximum bearable value, the amount of vegetable mass, after which it decreases, negatively influencing the efficiency of exploitation on pasture. Reducing the pressure of grazing on the vegetal carpet by improving the management of production systems, using staggered calving, is an alternative with beneficial effects on the vegetal carpet, through soil rotation and the multiple possibilities of restoring the vegetal mass. The use of meadows sown with white clover mixed with *Lolium perenne* at 12.5 cm or 25 cm spacings brings production increases of 20-22%, growth determined by the development of secondary and tertiary stolon mass and Phyto mass production and obtaining average milk production per lactation of 77.92+2.99 kg in the Turcan breed exploited on such meadows without the intake of concentrated feed during lactation.

In the case of free-range exploitation, in different production systems, the consumption of green mass through pasture varies between 58.0-76.0% due to:
- the floristic composition of the meadow;
- the maturity degree of the plants;
- the vegetation period and the amount of dry matter.

Free grazing on natural or artificial meadows is an essential part of sheep's life and the herd must be optimized and protected from the action of toxic plants that have adverse effects on health, with effects on food assimilation and health status. Changes in farm management, production specialization, reproductive control through hormonal stimulation and pushing calving in February allow farmers to take advantage of the free nutrients available on pasture and natural hay. Grazing control methods are intended to avoid negative impacts on the pasture by:
- well-managed grazing according to the development of the vegetative mass and the optimization of livestock;
- controlled grazing.

Well-managed grazing on fenced plots preserves vegetation and helps restore the vegetation if good practices include:
- an optimal number of maximum 4 UVM/ha of meadow;

- avoiding overgrazing by increasing the number of UVM;
- rotation on plots after a period of 35 days;
- fertilizing plots and irrigating dormant ones;
- the use of new managerial practices:
a. the use of breeds with a high degree of feed assimilation;
b. increasing the feed conversion capacity;
c. the use of microorganism that favors the fermentation of feed before ingestion.

The efficient use of food resources, the efficient management of production planning according to the state of vegetation of pastures, fodder and cereal crops, will reduce the total costs of maintaining production throughout the year. These changes of a managerial nature are: coordinating the accumulation of body mass with the maximum productivity of the meadows, the use of breeds that efficiently exploit the vegetative mass, the staggering of calving according to the evolution of the vegetative mass and the stimulation of lactation to reduce costs per kilogram of meat produced.

For stable operation considered the most efficient in the assimilation of resources, if it is economically managed, we recommend, through good nutrition practices, the administration of single chopped fodder for fattening, composed of the following percentage parts: fibrous 66.00%, silage 18.5%, corn 15.5%. The rations must be supplemented for balance with amounts of concentrates and vitamin-protein-mineral supplements, as well as lumps of salt for licking and drinking water will be provided at discretion.

In the case of intensive grazing of sheep in professional farms, to reduce the effects of overgrazing, good production practices must provide for:
- reducing the impact on environmental factors through risk management measures;
- optimizing the load of sheep according to the nutritional value of the vegetal carpet and its regeneration possibilities of the natural or artificial vegetative mass;
- the use of meadows that produce at least 6 tons of green mass per hectare for obtaining meat on pasture.

Taking into account these wishes, we recommend the implementation of new good nutritional practices for professional sheep farms of the Turcana breed in Arad County:
a. extensive exploitation managed on pasture;
b. a new system of modern sheep exploitation, through the management of mowed fodder, which will reduce the pressure on the plant resource, through:
- the development of integrated sheep farms;
- fodder production in agricultural crops;
- production of green mass on pastures by mowing;
- well-managed grazing to maintain a balance of vegetative mass-number of animals/ha;
- transition to ecological agriculture;
- the cultivation of fodder plants with high productions of vegetative mass;
- maximize profits by controlling food quantities;
- improving the management of meadows;
- administration of phosphorus fertilizers to increase the proportion of nitrogen fixing vegetables;
- controlled use of organic fertilizers.

This best practice system cannot be effective even if professional sheep farms have implemented the best integration management of milk or meat production, without the development of integrated farming systems that combine:
- the production of green mass with the consumption needs and the economic results predicted according to the genetic value of sheep;
- the production management methods in operation according to the new system implemented with the module administration of green mass supplemented with concentrates for energy and protein balancing of rations, for expressing the genetic value of the biological material as close as possible to its potential.

By implementing this new system of intensive exploitation of sheep through good nutritional practices implemented in production, the culture of fodder plants can be integrated with intensive exploitation, using human resources with a high degree of qualification throughout the year and additional income, the purpose of this integrative process being of:
- ensuring additional and complementary relationships, for the use of secondary products of one component of the agricultural system as input for another;
- maintenance of soil fertility;

- protection of natural environmental factors by reducing environmental risk;
- obtaining maximum profit per hectare;
- improving ecological diversity;
- preservation of floristic biodiversity.

Such modern sheep meat and milk production systems are also proposed by Integrated Farming System which suggests four categories of integrated farming systems that can be used according to the environmental conditions of the area in which is located the professional farm, of which only one (system B) includes sheep, which have a high degree of adaptability to the new exploitation system and ensure income throughout the year.

Good nutritional practices regarding the provision and administration of food to sheep depending on the specialization of production, also contribute to the improvement of their assimilation both in the stable and on the pasture. Because lactation plays a fundamental role in the nutritional intake of sheep: lactating sheep consume much more than other sheep. However, a highly variable correlation between feed intake and milk production was observed:

- the ability to assimilate food after calving does not automatically lead to an increase in milk production;
- the lactation period is the phase in which the sheep mobilize fat reserves to satisfy increased nutritional requirements.

For these reasons, it is stated that the good nutritional practices should consider through the management methods of nutrition:

- the physiological tendency to mobilize as many fat reserves as possible;
- restoring reserves after weaning or during pregnancy.

We believe that for large and medium-sized professional sheep farms, the most effective and safest managerial method for improving assimilation is:

a. administration of mowed green mass;
b. grazing on permanent meadows, for access to quality green mass under the nutritional ratio and assimilation index;
c. the grazing management methods used;
d. good grazing practices:

- continuous grazing, through the uninterrupted use of a grazing area;
- grazing by rotation, to restore the pasture.

We propose the implementation for professional farms, depending on the age category, of the following types of rotational grazing for meadows sustainability:

• grazing on fenced lands, where the area where grazed is adjusted according to the daily needs of the sheep category;
•preferential grazing, where lambs have access to better quality pastures;
•"leaders and followers" type grazing, in which the sola is grazed by leaders (lactating sheep) with higher requirements and then by followers (barren sheep) with lower needs.

CONCLUSIONS

In order to maintain the sustainability of pastures and reduce the pressure of different categories of sheep on plant resources, we recommend for professional farms, new ways of good nutritional management practices both for the exploitation system in stables and on pastures, for the production of meat and milk. In this sense, it is proposed to manage the feed mowed at the manger, which reduces the pressure on the pasture and contributes to the development of integrated farms in the production of food in agricultural crops which have in their composition *Trifolium repens*, *Lolium perenne* and from pastures through mowing, but also through economically well-managed grazing, in order to maintain a balance it is at least 5 tons of green mass per hectare at 4 UVM, between the capacity of pasture production and sheep load per hectare.

Obtaining sufficient productions, 2.5 tons per hectare of dry matter (SU) or over 6.0 tons of vegetative, mass to ensure the nutritional needs of sheep can only be achieved by improving the floristic composition of *Trifolium repens* and *Lolium perenne*, of the meadows and increasing their nutritional value, stimulating fertilization with phosphorus fertilizers for the development of nitrogen-fixing species or with organic fertilizers for increasing the production of green fodder.

Good nutritional practices, such as the use of ruminal symbionts, which break down the feed and make it easily digestible when fed with single feed in the stable, the selective breeding of sheep according to the production system, with good assimilation capacity and good conversion indices, the use in the pasture composition of plants with high digestibility *Trifolium repens* or *Lolium perenne* and the use of chopped fodder, implemented in professional sheep farms will contribute to the improvement of feed assimilation because there is a positive correlation between the intake of nutrients and the milk and meat productions achieved regardless of the production system. The most reliable method of improving feed assimilation is the administration of the green mass mowed at the manger and well-managed grazing on the permanent meadows. The grazing methods well managed and by rotation, controlled with plot grazing, preferential, or leader and follower grazing proposed to improve sheep productions in professional farms, allow the improvement of feed assimilation, through good nutritional grazing practices at the maturity of plant resources, continuous grazing and rotational grazing, with its three types that involve grazing in periods interpose with breaks to allow natural recovery or artificial of the vegetal carpet of the pasture.

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ANTIMICROBIAL ACTIVITY OF NATURAL CARBOHYDRATE POLYMERS AND THEIR MECHANISM OF ACTION - A REVIEW

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Abstract

Microbial infections represent a concerning health issue, and antibiotic-resistant pathogens represent a priority all over the world, having a negative impact on life quality and the economy. Therefore, there is an increase in focus on natural and renewable resources capable to inhibit microbial proliferation and their adherence on the surfaces. The mechanism involved in antimicrobial action and plant polysaccharides is still not clear. In this study, the plant polysaccharides extracted from different organs of plants, extraction methods, antibacterial and antiviral spectra and their mechanisms were reviewed. This review aims to provide a screening that could enhance the application of polysaccharides extracted from plants as antimicrobial agents related to the urgent need for natural alternative approaches to combat antimicrobial drug resistance (AMR).

Key words*: AMR, antioxidants, pathogens, plants green polymers, polysaccharides.*

INTRODUCTION

With the increasing incidence of antibiotic-resistant microorganisms and with the existence of a few viable remedies, potential preventive techniques, and a limited number of antibiotics, there is a necessity for the discovery of innovative medicinal approaches and natural antimicrobial therapies, to fight the emerging antimicrobial-resistant threat. Microbial biofilms make infection control more complex; the horizontal gene transmission of resistance is facilitated by extracellular DNA present in the biofilm matrix. In humans, approximately 80% of all microbial infections are a direct result of biofilms (Singer et al., 2016, Asghar et al., 2021, Uddin et al., 2021, Irfan et al., 2022). WHO 2021 mentioned that Antimicrobial Resistance (AMR) represent one of the top ten global public health threats facing humanity and published a list in 2017 with priority pathogens for which are needed new antibiotics (the first and critical priority is: *A. baumannii, P. aeruginosa, and Enterobacteriaceae*; the second and critical priority are: *E. faecium, S. aureus, H. pylori*, *Campylobacter* spp., *Salmonellae, N. gonorrhoeae* and on the third and medium priority are: *S. pneumoniae, H. influenza and Shigella* spp.), and antivirals including antiretroviral (ARV) drugs.

In the soil were also found antibiotic-resistant microorganisms. The microbiota of the soil is very important for life on our planet, having a role in the cycling of carbon, nitrogen and other essential nutrients (Armalytė et al., 2019). The usage of conventional agricides influences the microbial activities in soils and contributes to the increase in incidences of co-resistance. There are a lot of reports on the effect of biocide impacting the development of cross-to-co-resistance in pathogenic bacteria and also the agricultural usage of antibiotics and its impact on soil microbiome that can lead to antibiotic resistance (Paul et al., 2019; Udiković-Kolić et al., 2014). Resistant microorganism as *Pseudomonas* spp., *Stenotrophomonas* spp., *Sphingobacterium* spp., and *Chryseobacterium* spp. were found in the soil, their resistance being dependent on the efflux mechanisms and on specific transporters (Armalytė et al., 2019).

Publications related to natural polysaccharides are increasing year by year (Figure 1), polysaccharides received more and more attention, having been explored from natural resources due to their multifaceted properties like antimicrobial, antioxidant, antidiabetic (having hypoglycemic action), anti-inflammatory, anti-angiogenesis, immunomodulatory (can modulate innate immunity), anticancer, anti-angiogenesis, anticoagulant, antihyperlipidemic, antihepatotoxic, anti-ageing, prebiotic, probiotic and symbiotic action, regulating intestinal microbiota. Polysaccharides are biomolecules that act as a major role in the formulation of pharmaceuticals (drug delivery applications due to their solubility, permeability, and diffusivity), foods (natural excipients in the form of thickening, binding, gelling, suspending, emulsifying, stabilizing, film forming, matrix-forming, and disintegrating agent), health products (tissue engineering, abrasion curing, drug distribution, biosensors), fabric, dyes/paints, paper, gums/binding agents (Miteluț et al., 2015, Friedman, 2016, Rahimi et al., 2020, Ghosh et al., 2021, Misaki et al., 2021; Otu et al., 2021, Sindhu et al., 2021, Albuquerque et al., 2022, Bai et al., 2022; Pan et al., 2022, Sharma et al, 2022, Sun et al., 2022, Ray et al., 2023). A potential use of polysaccharides obtained from fungi (especially those that have immunomodulatory and antioxidant activities) is for vaccine production due to their low-cost source (Barbosa & Carvalho, 2020).

Polysaccharides are not only vital biomacromolecules but also environmentally safe products which have in their structure homo or hetero monosaccharides chains and uronic acids connected with glycosidic bonds, their configuration being influenced by their source (Otut et al., 2021). The green synthesis of plant carbohydrate polymers is preferred for the following aspects: improving biocompatibility, avoiding the utilization of toxic organic chemicals, and reducing the cost (Asghar et al., 2021). The most common polysaccharides and their sources are as follows: bacteria (exopolysaccharides, capsular polysaccharides, lipopolysaccharides, peptidoglycans, teichoic acids); algae (sulfated polysaccharides: laminarans, fucoidans, carrageenans, ulvans agar, sodium alginate), fungi and yeast (exopolysaccharides, chitin, glucans, galactomannans), mushrooms (β-glucans polysaccharides, α-glucuronoxylomannans, mannogalactan), lichens (galactoglucomannan, sulfated polysaccharides), plants (inulin, pectin, gums, cellulose, hemicelluloses, arabinans, xylans and starch), insects (chitin and its derivatives) and animals (glycogen, hyaluronic acid, glycosaminoglycans, heteropolysaccharides, sulfated glycosaminoglycans, keratin sulfate, heparan sulfate, dermatan sulfate, and chondroitin sulfate) (Friedman, 2016; Ullah et al., 2019; Barbosa & Carvalho 2020; Liu et al., 2020b; Valasques Junior et al., 2020; Sindhu et al., 2021; de la Harpe et al., 2021; Bai et al., 2022; Ray et al., 2023).

Figure 1 presents the number of papers listed by Science Direct since 2000 using "polysaccharides, plant polysaccharides and antimicrobial plant polysaccharides" as search terms.

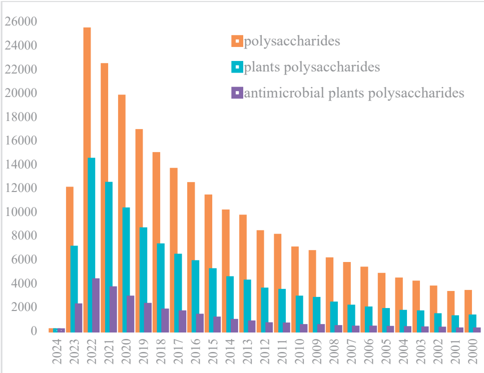

Figure 1. The evolution of the articles from 2000-2024 related to polysaccharides from Science Direct

The activities of plant polysaccharides are influenced by the number of branches that they contain, a greater number of branches or side chains in fucose, galactose, and/or mannose are associated with a high immunostimulatory activity that is in close connection with antimicrobial activity, according with Zhou et al., 2022.

Polysaccharides' molecular weights are related to their antimicrobial, immunostimulatory, antioxidant activities and phenolic acids content, (1→4)-d-mannans exhibit high immunostimulatory activity compared to low molecular weight (1→4)-d-mannans, acidic polysaccharides with high molecular weights are more active than those with lower molecular weights, instead for carrageenans, low molecular weight fractions are associated to higher immunostimulating properties (Bai et al., 2022).

Table 1. Antimicrobial activity of plants and polysaccharides

Plant name	**Family**	**Antimicrobial activity**	**References**
Daucus carota peel by-product (water-soluble polysaccharides)	Apiaceae	*E. coli*, *S. enterica*, *Enterobacter* sp., *S. aureus* and *Micrococcus luteus*	Ghazala et al., 2015, Albuquerque et al 2022
Periploca laevigata- root barks	Apocynaceae	*E. coli*, *P. aeruginosa*, *L. monocytogenes*, *S. aureus*, *M. luteus*, B. cereus	Hajji et al., 2019, Zhou et al., 2022
Ilex paraguariensis- leaves and stems	Aquifoliaceae	*E. coli*, *E. cloacae*, *S. enteritidis*, *S. typhimurium*, *B. cereus*, *M. flavus*, *S. aureus*, *L. monocytogenes*	Kungel et al., 2018, Li et al., 2022, Zhou et al., 2022
Panax ginseng- roots *(*polysaccharides PG-F2 (12 kDa) and PG-HMW (80 kDa)	Araliaceae	*S. aureus*, *B. pumilus*, *B. subtilis*, *H. pylori;P. gingivalis* and *A.actinomycetemcomitans* adhesion	Lim et al., 2002, Zhao et al., 2019, Zhou et al., 2022
Carthamus tinctorius-bee pollen (PBC-II)	Asteraceae	*E. coli* and *S. aureus*	Wu et al., 2021, El-Ghoul et al., 2021
Tridax procumbens	Asteraceae	*Vibrio alginolyticus* and *Vibrio harveyi*	Naqash &Nazeer., 2011, Zhou et al., 2022
Saussurea controversa- leaves (polysaccharides of 5.5 kDa) *Tamarindus indica*- seeds	Asteraceae Leguminosae (Fabaceae)	*S. aureus*	Khlusov et al., 2019, Albuquerque et al 2022, Zhou et al., 2022
Epimedium acuminatum- leaves	Berberidaceae	*E. coli*, *B. subtilis*	Cheng et al.,2013, Zhou et al., 2022
Cordia myxa- fruits	Boraginaceae	*E. coli*, *P. aeruginosa*, *B. cereus*, *S. aureus*	Hoijati & Beirami-Serizkani, 2020, Zhou et al., 2022
Lepidium sativum -seeds	Brassicaceae	*E. coli*, *S. aureus*	Alkahtani et al., 2020, Zhou et al., 2022
Pea pod- Byproducts	Fabaceae	*P.aeruginosa*, *B.subtilis*, *B. thuringiensis*, *M. luteus*	Belghith-Fendri et al., 2018, Zhou et al., 2022
Trigonella foenum-graecum- seeds-Senegrain Water-Soluble Polysaccharide (SWSP)	Fabaceae	*B. subtilis*, *S. enterica*, *M. luteus*	Ktari et al., 2020, Zhou et al., 2022
Hypericum perforatum- leaves	Hypericaceae	*S. dysenteriae*, *S. typhimurium*, *E. coli*	Heydarian et al., 2017, Zhou et al., 2022
Aloe barbadensis (leaf gel)	Liliaceae	*E. coli* and *E. faecalis*	Salah et al., 2017, Sindhu et al.., 2021
Lilium davidii var. unicolorCotton-bulbs	Liliaceae	*P. putida*, *K. pneumoniae*, *B. cereus*, *M. luteus*	Hui et al., 2019, Zhou et al., 2022
Althaea officinalis-roots (polysaccharides- 4.87 × 104 Da)	Malvaceae	*E. coli*, *S. enterica*, *S. aureus*, *B. circulans*	Abbaspour et al., 2022, Zhou et al., 2022
Durio zibethinu- fruit rind, peel	Malvaceae	*A.actinomycetemcomitans*, *S.mutans*, *Vibrio harveyi S.aureus* , *B. subtilis*, *B.aeruginosus*, *E.coli*, *S.typhi*, *P.aeruginosa*, *C. albicans*	Wu et al.,2010, Thunyakipisal et al., 2010, Sutanto et al.,2022, Zhou et al., 2022
Malva aegyptiaca- leaves (precipitation with cetylpyridinium chloride, precipitation with ethanol)	Malvaceae	*E. coli*, *K. pneumoniae*, *S. enterica, S. typhi*, *M. luteus*, *S. aureus*, *B. cereus*	Fakhfakh et al., 2017, Zhou et al., 2022
Forsythia suspensa- fruit	Oleaceae	*E. cloacae*	Liu et al., 2020 a, Zhou et al., 2022
Olea europea - leaves	Oleaceae	*S. enterica*, *M.luteus*	Khemakhem et al., 2018, Zhou et al., 2022
Zizyphus jujuba (galacturonic acid 242 kDa)	Rhamnaceae	*E. coli, B. subtilis, S. mutans*, *P. gingivalis*	Feng et al., 2021, Xu et al., 2022 b
Crataegus azarolus var. aronia-pulps,seeds	Rosaceae	*E. coli*, *K. pneumoniae*, *S. typhimurium*, *B. cereus*, *L. monocytogenes*, *S. aureus*, *E. faecalis*, *P. aeruginosa*, *Listeria monocytogenes*, *B. cereus*	Rjeibi et al., 2020, Albuquerque et al 2022, Zhou et al., 2022
Camellia sinensis- leaves, *Tetrastigma hemsleyanum* Diels et Gilg's (TDG)	Theaceae Vitaceae	*E. coli*	Chen et al., 2019, Zhu et al., 2020, Zhou et al., 2020, Zhou et al., 2022
Polygonatum cyrtonema Hua-rhizome, *Allium cepa*- bulbs, *Broussonetia papyrifera-leaves* *Peony*- seed dreg	Asparagaceae Liliaceae Moraceae Paeoniaceae	*E. coli*, *S.typhimurium*, *S. aureus*, *B. subtilis*, *P. aeruginosa*	Han et al.,2016, Li et al., 2018, Li et al., 2018 b., Ma et al., 2018, Xu et al., 2022 a, Zhou et al., 2022
Taraxacum officinale- shoots *Salicornia arabica*- leaves, *Cucurbita moschata*- seeds (Polysaccharides-21,000 g/mol) *Cyclocarya paliurus*- leaves, *Phyllostachys pubescens*- leaves (5.77 × 103 Da),	Asteraceae Chenopodiacea e Cucurbitaceae Juglandaceae Poaceae	*E. coli*, *B. subtilis*, *S. aureus*, *C. albicans*, *F. phyllophilum* and *F. oxysporum*	Qian, 2014, Hammami et al., 2018, Xiao et al., 2020, Xie et al., 2012, Albuquerque et al 2022, Li et al., 2022, Zhou et al., 2022,

Low molecular weight (3.105×10^4 Da) polysaccharides produced by *Chaetomium globosum*, had higher inhibitory effects against *E. coli* and *S. aureus* than high molecular weight (5.340×10^4 Da) (Zhang et al., 2021). Álvarez-Viñas 2021, and co-workers found that sulfated polysaccharides have a weak ability to tissue-penetrating, making it almost impossible to pass away the cell membranes, making them suitable for external use. The monosaccharide composition could also influence antimicrobial activity (Albuquerque et al., 2022). Because of their ability to enter in the cells due to reduced molecular weight, low viscosity, and high solubility at neutral pH, oligosaccharides may have stronger antiviral effects (Álvarez-Viñas et al., 2021). Ketones or aldehydes with many hydroxyl groups make up monosaccharides (Barriga & Fields 2023). 11-hydroxy-16-hentriacontanone, isolated from leaf cuticular wax of *Annona squamosa*, has antibacterial and antifungal activity on both Gram-positive and Gram-negative bacterial strains (Shanker et al., 2007). Hellewell and Bhakta 2020 describe in their study how the chemical classes of ketones, chalcones and stilbenes have the potential to help in the fight against antimicrobial resistance of *E. coli*, *B. subtilis*, *M. smegmatis, M. aurum* and *M. bovis* BCG, being novel efflux pumps and biofilm inhibitors. Different polysaccharides derived from plants such as aloe vera, okra fruit, ginseng, liquorice root, and blackcurrant have been demonstrated to prevent adherence of *Helicobacter pylori* onto the mucin and gastric cells in vitro (Ray et al., 2023). Extracted polysaccharides from *Broussonetia papyrifera* fruits were further purified to obtain three different fractions of the polysaccharides with antimicrobial activities: BPP-1 (314.4 kDa), BPP-2 (284.2 kDa), and BPP-3 (235.4 kDa). Bamboo leaves polysaccharides, with 60.23% xylose content showed the best antimicrobial activity. Purified fraction of *Epimedium acuminatum* Franch. polysaccharides with major monosaccharides glucose and galactose showed the best antimicrobial activity (Otu et al., 2021) The therapeutic efficiency in some disorders can be increased by controlling the particle size that range between 10-500 nm (Zhao et al., 2019).

As a result, these polysaccharides-based nanomaterials can be effectively employed as a carrier to transport antibiotics, genes, or immunotherapeutic antigens, acting as a nano-vaccine. (Ahmad et al., 2022). Guar gum hydrogels as an injectable pharmaceutical form, can exhibit rapid self-healing, and antibacterial properties towards: *E. coli*, *P aeruginosa*, *S.aureus* thereby confirming new and optimistic prediction for injectable hydrogels (Talodthaisong et al., 2020).

EXTRACTION METHODS

According to Otu et al. (2021), extraction solvents has been found to play a mechanistic role in antimicrobial activity. Polysaccharides from both natural and modified sources were found to be insoluble in organic extraction solvents, due to their hydrophilic property. Therefore, water is the most common solvent and the base solvent for extraction methods, including acid and alkali extraction, and enzymolysis methods (Albuquerque et al., 2022).

The use of **acidic solutions** with temperature, time, and pH-controlled has the main advantage of a high extraction rate and short extraction time. Similar to the acid extraction method mentioned above, the alkaline extraction method may also remove polysaccharides from plants by destroying their cell walls (Talodthaisong et al., 2020). The main disadvantages are modifications in the polysaccharide structure, organic solvents mainly alcohols can selectively precipitate some target carbohydrates whilst other components remain dissolved in the extraction mixture. The high viscosity of the extract obtained can compromises filtration steps, and also its flavor, affecting also the quality and color of the polysaccharides (Albuquerque et al., 2022; Otu et al., 2021).

The disadvantages of **enzymolysis** extractions are related to the price of the enzymes, and the limiting factors (enzyme mass concentration, temperature, pH) associated with a large-scale application (Albuquerque et al., 2022).

Enzymatic hydrolysis of polysaccharides and transglycosylation can be the focus of a new research field that aims to increase efficiency and decrease the production costs of functional oligosaccharides (Martins et al., 2023)

Ultrasonic extraction has the advantages of high extraction efficiency, short time, and low energy consumption, while the main disadvantage is the difficulty to reach the best frequency, solid-liquid ratio, and temperature conditions. Other extraction methods have occasionally been applied in scientific researches as: high voltage pulsed electric field, ultrahigh pressure, microwave, liquid phase pulse discharge, supercritical carbon dioxide and subcritical water extraction methods according to Albuquerque et al., 2022.

Cellulose is an inexhaustible raw material that exists as the most abundant polysaccharides present primarily in lignocellulosic material (Ma et al., 2021). As a biomaterial, cellulose offers numerous advantages such as nontoxicity, high availability, renewability, low cost, biodegradability and other quintessential physical and chemical properties (de la Harpe et al., 2021; Mayer et al., 2021). Cellulose is low soluble in most organic solvents, in water being insoluble, these properties represent the most important and difficult step in extraction. This is due to its stiff molecules (high degree of polymerization ranging from 10,000 glucopyranose units and numerous intra- and intermolecular hydrogen bonds (Taokaew et al., 2022). Physical toughness is also enhanced by hemicellulose, which is responsible for binding cellulosic fibres to lignin (Przypis et al., 2023). Wu et al. (2023) developed a new method for cellulose **derivatization using amino acid hydrochloride** ([AA]Cl) because it can efficiently catalyze esterification reactions and can break the hydrogen bond network, to synthesize cellulose amino acid. The synthesized products demonstrated bacteriostatic activity on *E. coli* and *S. aureus*. Mayer et al., 2021, in their study, mentioned that using **selective cleavage of the C2 single bond and C3 bond by periodate oxidation**, leads to the formation of a very important cellulose derivative, 2,3-dialdehyde cellulose. These aldehyde groups are sensitive to Schiff base reactions, having antimicrobial activity on *E. coli* and *S. aureus*. Different derivatives of cellulose such as oxidized regenerated cellulose, hydroxyethyl cellulose and cellulose nanofibrils, are ideal candidates for new, innovative sutures (de la Harpe, 2021). Ahmad et al., 2022, revealed that to produce cellulose nanofibers with antimicrobial activity, the most suitable method is **hydrolysis through acid**. Cellulose nanofibers obtained throught hydrolysis, enhance gut health by increasing good bacteria from *Lactobacillaceae* while decreasing *Streptococcaceae* in mice. *Streptococcaceae*, has been associated with obesity, diabetes, as well as colon cancer. Ilangovan et al., 2018, extracted cellulose fibres from *Curcuma longa* L. leaves and stalks, with alkali solutions and demonstrated that these natural cellulose fibers have inhibition activity on *S. aureus*, *E. coli* and *B. cereus*. However, the poor solubility of cellulose in water and other common solvents has prevented its full utilization in the biomedical and related fields, new cellulose production techniques are emerging, such as dissolution and functionalization in ionic liquids known as green extraction methods (Taokaew et al., 2022).

Hemicellulose is composed of two or more different monosaccharide units, hexoses (β-D-glucose, β-D-mannose, and β-D-galactose), pentoses (β-D-xylose and α-L-arabinose), fructose, rhamnose in lower quantities and uronic acids, β-(1→3, 1→4)-glucans receiving little consideration (Zhang et al., 2021b).

The antimicrobial activities of hemicellulose are less studied as against cellulose and lignin. Hemicellulose films can inhibit the growth of the bacteria *S. aureus*, *E. coli*, and *P. aeruginosa*. Hemicellulose extracted from almond gum, have proven higher inhibitory activity against *B. thuringiensis*, *S. enterica*, and *P. aeruginosa* and moderated inhibition against *Sal. thyphimirium*, *Actinomyces sp.*, *K. pneumonia*, *L. monocytogenes*, and *B. subtilis* (Lobo et al., 2021).

Lignin is composed of C_6-C_3 phenylpropane units including sinapyl, coniferyl and p-coumaryl alcohols, or syringyl, guaiacyl and *p*-hydroxyphenyl units, the unit ratio in lignin varies according to biomass species in terms of the number of methoxy groups (Lu et al., 2022). The polyphenolic structure of lignin in addition to the presence of O-containing functional groups (including phenolic hydroxyl, carbonyl, carboxyl, and methoxy groups) is potentially responsible for antimicrobial activities (Greco et al., 2019). For lignin was used extraction methods such as:

- **Kraft lignin** (it is used a solution of NaOH and NaHS, at a temperature between 150 to 170°C);
- **Hydrolysis lignin** and **organosolv lignin**, use organic solvents (acetic acid, ketone, and ester), the organosolv lignin being highly pure and sulphuric-free;
- **Ionic liquids extraction**, which uses green solvents has high thermal stability and present low toxicity. On enzymatic hydrolyzed lignin from corn stalk, it was observed that in the first extraction showed higher antibacterial activity against all the bacteria *E. coli*, *B. subtilis*, *Sal. enterica* and *S. aureus*, Gram-positive microorganisms being more sensitive to the lignin extract than the Gram-negative bacteria. In this context, the materials produced with lignin can be considered for different areas of application, as a natural antimicrobial agent (Lobo et al., 2021). Salem et al., in 2014 showed that lignin extracts show antimicrobial activity against *Listeria innocua* and *Bacillus sp.* than against *Klebsiella sp.* The antimicrobial mechanism is given by sugar content of the lignin that might cause and/or support the adhesion to the bacterial membrane. The activity against *S. aureus* can be increased by the interaction between peptidoglycan layer of bacterial cell walls and sugar molecules. Lignin films present activity on Gram-positive bacteria more than Gram-negative bacteria. Films containing organosolv lignins presented higher antimicrobial activity on *S. aureus*, than kraft lignins. For *E. coli*, the kraft lignin films presented no activity at low concentration. These findings that polysaccharides actions are more efficient against Gram-positive bacteria than Gram-negative ones could be related to the presence of an external phospholipid membrane in Gram-negative bacteria, limiting the diffusion of hydrophobic compounds through its lipopolysaccharide cover, and acting as a barrier against hyper acidification, according to Albuquerque et al., 2022.

Pectin it is a naturally occurring complex heteropoly saccharide with a cytocompatible mechanism, consisting of galacturonic acid and low amount of neutral sugars in the side chains, being considered one of the most complex macromolecules in nature (Ciriminna et al., 2020; Lisitsyn et al., 2021). Pectin has a large broad-spectrum antimicrobial capable to kill Gram-negative bacteria, yeasts and non-filamentous fungi. Its mechanism of action involves the binding action of the carboxylic acid groups in the main backbone of the biopolymer, enhances mechanical properties and decreasing the solubility of water. Pectin being a good source for the development of nanoparticles. Optimal antibacterial activity was observed at acid pH (around pH 5-6) (Ciriminna et al., 2020; Lisitsyn et al., 2021; Albuquerque et al., 2022).

Processes such as **microwave hydro diffusion and gravity and hydrodynamic cavitation carried out with ultrasounds** applied to the wet peels of different fruits (apple, citrus, *Opuntia-ficus indica* etc.) using water as solvent, followed by freeze-drying, allow pectins embedding polyphenols, flavonoids, terpenes and phenolic acids. The pectin obtains through the methods described above, shows high antibacterial activity against both Gram-positive and negative bacterial strains *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *H. pylori*, *E. coli*. 1% pectin solution killed within the first 15 min. of contact more than 90% of the Gram-negative bacteria (*S. vulgaris*, *S. typhi*, *S. paratyphi*, *S. typhimurium*, *K. aerogenes, E. coli*, *P. vulgaris*, *B. bronchiseptica* and *P. aeruginosa*). Pectin's **green extraction** methods represent a new and highly promising area of research in the life sciences and medicine (Ciriminna et al., 2020, Lisitsyn et al., 2021). Lin et al., 2023, obtained high molecular weight polysaccharides (>84.7 kDa) from citrus peel pectin from aqueous extract of 9 different citrus species through enzymatic methods. Those citrus peel pectin oligosaccharides presented antimicrobial activity on: *S.epidermidis*, *B.subtilis*, *S.aureus*, *K. pneumoniae*. Another mechanism of pectin action on pathogen microorganisms is through prebiotic activity, reducing pathogens' growth in favor of friendly microorganisms (Albuquerque et al., 2022). Instead Lisitsyn et al., 2021, found that bioactive packaging films made from pectin have very weak antimicrobial properties.

Another extraction for polysaccharides is the one that uses **dilute alkali** solutions. Onion polysaccharides extracted in this way had the highest antibacterial activity against both Gram-positive and Gram-negative bacteria.

From *Lilium davidii* var. unicolor were extracted two heteropoly saccharides, BHP-1 and BHP-2. C**etylpyridinium chloride was used to precipitate polysaccharide** in order to obtain BHP-1, and BHP-2 was obtained by **precipitating ethanol**. The inhibitory zone of BHP-1 was larger, which may be related to the high sugar content and high number of sulfate groups (Zhou et al., 2022).

Methanol, ethanol and hydroalcoholic solvents are very good to separate ketoses from non bioactive aldoses. To confirm the mechanistic role of organic solvent (alcohol), a study on ethanol extract of *Phellodendron amurense* bark showed better antimicrobial activity than the aqueous extract.

Anisophyllea laurina pulp and seed powder were extracted by sonication over an ice bed with methanol/water 80:20 (v/v), ethanol/ water 80:20 (v/v), ethyl acetate/water 1:5 (w/v), and finally with water for 15 mins. The ethanol and methanol extracts recorded the highest phenolics, flavonoids, tannin, and monomeric anthocyanin content. Ethanol and methanol seed extract showed best antimicrobial activity on both gram negative and gram-positive bacteria, ethyl acetate seed extract showed moderate antibacterial effect and water extract doesn't have antibacterial effect. This result displayed the importance of plant bioactive compounds solubility in an extraction solvent and its strong correlation with antimicrobial activity (Otu et al., 2021). Darmohray et al, 2021 observed also that 20% aqueous extract of *Galega orientalis* has a slight antimicrobial effect on Gram-negative and Gram-positive bacteria and yeast.

Generally, it is difficult to obtain pure polysaccharides by performing only one method, thus combined techniques have been employed to improve purification and achieve good yields Albuquerque et al., 2022.

ANTIMICROBIAL MECHANISMS

Until now, the mechanism of polysaccharide antimicrobial activity was not been fully elucidated (Otu et al., 2021; Lin et al., 2023). However, the degree of polymerization, alteration, solubility and monosaccharide composition is likely to influence this mechanism while no consensus mechanism has been accepted by researchers (Lin et al., 2023). To withstand environmental and other drastic conditions, most of the pathogenic microbes create an exopolysaccharide biofilm around all growing populations, which protects the pathogenic microbes from many bacterio-phages, biocides, and immune cells from the host (El-Batal et al., 2020).

Bacterial cell membrane permeability

Gram negative bacteria *S. typhi* without the peptidoglycan wall may hydrolyze available polysaccharides in order to produce monosaccharides as source of nutrition. This property of the bacteria it is confirmed by *Capparis spinosa* leaf polysaccharides. A higher concentration led to a reduction in inhibition zone. Aqueous extract of *Syzgium aromaticum* seeds applied on *E. coli*, *P. aeruginosa*, and *S. aureus* lead to an increase in malondialdehyde which may cause peroxidation of the lipid bilayer of the bacterial cell membrane and subsequently led to the release of nucleic acid content. The lipophilic character of polyphenolic compounds favors the interaction with the cell membrane which enhances their antimicrobial activity. Their interactions with cell membranes may induce irreversible damage to the cytoplasmic membrane and coagulation of the cell content and even lead to the inhibition of intracellular enzymes (Otu et al., 2021).

Aqueous polysaccharides extract from *Trachyspermum ammi* presented more mannose than glucose, and showed better antimicrobial activity than *Dolichos biflorus* which had more glucose than mannose. This observation suggested the presence of mannose receptor in the targeted microorganism membrane and therefore aided in the higher absorption of polysaccharides (Otu et al., 2021)

Albuquerque et al., 2022, suggested that the polysaccharides could induce the disruption of the cell wall of bacteria and increase ion permeability and the antimicrobial activity could be related to their total sugar contents.

The plant polysaccharides may interact with the cell membrane through **hydrophobic action and electrostatic adsorption, or glycoprotein receptors**. There is hydrophobic–hydrophobic interaction between the hydrophobic substrates and lipid bilayers of bacterial membranes.

The protein or polyphenols in some plant polysaccharides confer **hydrophobic characteristics** to these polysaccharides, as apple pomace polysaccharides that can diffuse passively through the lipids of cytoplasmic membrane bilayer into the bacterial cytosol, leading to the escape of intracellular components and alteration of the bacterial enzyme system.

Electrostatic adsorption

Plant polysaccharides display antibacterial activity by increasing membrane permeability via electrostatic adsorption, which can lead to a rapid increase in the amount of water-soluble proteins in cells, protein dissolution, DNA degradation, leakage of essential molecules, and cell death

Inhibition of the adsorption of nutrients

Plant polysaccharides can block the absorption of nutrients by bacteria and affect energy metabolism, leading to the inhibition of bacterial growth. Polysaccharides extracted from *Tetrastigma hemsleyanum* can block glycolysis and gluconeogenesis of *E. coli*. In this way, deprives bacteria in obtaining adenosine triphosphate and nicotinamide adenine dinucleotide necessary for energy metabolism (Zhou et al., 2022). Sulphated polysaccharides extracted from *Malva aegyptiaca* can also inhibit cell growth (Barriga & Fields, 2023). The antibacterial activity of polysaccharides might stem from the fact that they act as a barrier preventing the entry of nutrients. The mechanism of **metal toxicity** is metal species-specific. Some metals can cause protein dysfunction, lead to the production of reactive oxygen species (ROS) and depletion of antioxidants, impair membrane function, interfere with nutrient assimilation, and be genotoxic. Iron is an essential element for bacterial growth usually needed in a range of 0.4-4 μM.

Metals and bacterial cells

Yerba mate polysaccharides show antibacterial activity against various strains except *for E. coli*. The antibacterial activity of polysaccharides can be their ability to inhibit the absorption of iron by bacteria. *E. coli* can secrete enterobacteria, which has a high affinity for iron; consequently, the absorption of iron by bacteria is inhibited after the addition of plant polysaccharides (Kungel et al., 2018). According to hard-soft acid-base theory, soft acids such as Hg, Cu, Ag and Cadmium and borderline acids such Co, Ni, Cu and Zn do associate tightly with soft bases, such as sulfhydryl groups found in proteins. The antibacterial activity of these metals is therefore dependent on their affinity to sulfhydryl group (Otu et al., 2021).

Aqueous extract of *Tilia* leaves was used to reduce copper ions in sulfate pentahydrate solution (4:1 v/v) to form Cu nanoparticles. To reduce zinc ions (Zn^{2+}) to zinc oxide nanoparticles was used extract from *Brassica oleraceae* leaves. The use of chemical and biological techniques has shown that metal ions such as Cr (VI) As (III), and Te (IV), but especially Fe (II) and Cu (II) do increase ROS which leads to DNA damage and the inhibition of enzymes activity essential for cell growth.

The phenolic acids present in the carbohydrate polymers also can act as secondary anti-oxidants by inducing the activity of antioxidant enzymes such as peroxidase, catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase and glutathione S-transferase (Ghosh et al., 2021).

Plant polysaccharides can also **prevent the adhesion of pathogenic** bacteria to host cells. The *Panax ginseng* polysaccharides are not able to inhibit the adhesion of bacteria and cells such as *Lactobacillus acidophilus* and *E. coli*, suggesting that they have selective anti-adhesion activity (Ghosh et al., 2021).

Bacteria spores formed can protect and make organisms indestructible to anti-bacterial action. However, water-soluble polysaccharides of cabbage remarkably displayed great antimicrobial effect against a spore-formed gram-positive *Bacillus* spp. Curcumin extracted from rhizomes of *Curcuma longa* demonstrated the ability to inhibit *Clostridium difficile* formed spore. The anticlostridial properties of curcumin can be related to its phenolic content (Otu et al., 2021).

Liu et al. (2020 b) presented **antiviral activities** of several polysaccharides anti HSV from *Acanthopanax sciadophylloides*, *Adenanthera pavonina*, *Avena sativa*, *Azadirachta indica*, *Caesalpinia ferrea*, *Cedrela tubiflora*, *Echinacea purpurea*, *Portulaca oleracea*, *Prunella*, *Stevia rebaudiana*, having the following antiviral mechanisms: inhibits adsorption and penetration, virucidal effects, inhibits viral DNA and protein synthesis.

Cydonia oblonga, *Glycyrrhiza glabra*, *Nigella sativa*, *Tinospora cordifolia*, and *Zizyphus jujube* against COVID-19 because of their proven antiviral it has been suggested that sulfated polysaccharides can be used to bind the virus proteins, consequently blocking cell entry. An important member of the glycose amino glycans is heparin, which represents a key binding factor for SARS-CoV-2 and Ebola virus. Most physiological and pathophysiological activities of heparin sulfate are due to electrostatic interactions with various proteins. In investigations on the SARS-CoV-2 virus, heparin and its derivatives, such as enoxaparin, 6-O-desulfated UFH, and 6-O-desulfated enoxaparin, have shown encouraging results (Barriga & Fields, 2023) and on HSV-1, HSV-2, HPV-16 and RSV infections (Lu et al., 2021).

Glycosaminoglycans are proteins heavily decorated with sulfated polysaccharides. These proteins are cellular receptors for the binding of viruses like herpes simplex, HIV, dengue virus and many others (Barriga & Fields, 2023).

Ferula sinkiangensis polysaccharide, *Morus nigra* polysaccharide and their sulfated modifiers had the best antiviral effect on the anti-Newcastle disease virus. Polysaccharide from the *Adenanthera pavonina* seeds have antiviral action on poliovirus type 1. *Sophora tomentosa* root polysaccharide and its sulfate, significantly inhibited the protein translation and RNA synthesis of duck hepatitis A virus-1. The sulfate derivative of *Cyathula officinalis* polysaccharide showed significant antiviral effects by interfering with the HSV-2 adsorption process. Sulfated derivatives of Angelica polysaccharides showed effect on murine leukemia virus in vivo. Sulfated modification of *Polygonum taipaishanease* polysaccharide can notably increase the activity on: transmissible gastroenteritis virus, *Shigella* and *E. coli*. The crystal-like arrangement of the nano assemblies with a higher level of sulfation exerted stronger antiviral activity, indicating the polysaccharide configuration may be important in the development of antiviral agents (Lu et al., 2021).

CONCLUSIONS

In recent years, there has been shown an increase in researches about the extraction, characterization, and biological functions of plant polysaccharides.

The most used methods to obtain polysaccharides from plants were those by hot water extraction and alcohol precipitation. Microwave hydro diffusion and gravity and hydrodynamic cavitation carried out with ultrasounds are green extraction methods representing new and highly promising extraction methods, but the purification yield of plant polysaccharides can represent a limiting factor for large-scale commercial use.

Moreover, investigations of the shelf life of possible formulations based on polysaccharides will be very important and the investigations of their biocompatibility to highlight possible adverse effects. Thus, it is expected that in the future, after adequate testing in vivo these carbohydrates can be used in formulations with therapeutic and preventive solutions.

We hope that this review could promote the application of plant polysaccharides in antimicrobial formulations in the future.

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THE EVOLUTION OF THE FLORISTIC COMPOSITION OF AN ECOSYSTEM IN THE AREA OF SANDS IN SOUTHWEST ROMANIA UNDER THE INFLUENCE OF ENVIRONMENTAL FACTORS

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Abstract

The present work aimed to analyze the influence of climate change on the floristic composition of an ecosystem in the sandy area located in southwestern Romania, namely in the Dăbuleni area, Dolj county. The research was carried out on areas where the influence of the zoo-anthropogenic factor is very low or absent and showed that changes in floristic composition occurred within these ecosystems. The observations made allowed the creation of a floristic list that was compared with the literature. Although the number of species has been reduced, the herbaceous cover has nevertheless been well covered. The dominant species which give the physiognomy of these sites belong to the category of those which are not characteristic of sandy soils but which have adapted very well to these conditions. Acclimatization of other species, brought from other regions, has not been possible. Typical psammophilous species such as Polygonum arenarium, Mollugo cerviana, Silene conica, Tragus racemosus, Plantago arenaria, Jasione heldreichii, Achillea ochroleuca, Centaurea soltitialis ssp. solstitialis, etc., prefer open sites because they do not seem to tolerate competition with other species.

Key words: *spontaneous flora, change climatic, Oltenia, Romania*.

INTRODUCTION

Climate change affects ecosystems, altering vegetation composition and ecosystem functioning. Globally, long-term analysis of climate change and its biological effect has shown that it affects species physiology, species distribution, organism phenology, composition and biocenosis dynamics (Cosmulescu & Birsanu, 2018; Cosmulescu et al., 2020; Parmesan & Yohe, 2003; Grimm et al., 2013; Jennings & Harris, 2017; Buican et al., 2021a; 2021b). Urbanization and environmental change is one of the main causes of the ecosystem and biodiversity loss and alteration worldwide (Blouin et al., 2018; Ilie & Cosmulescu, 2003). However, species invasion and extinction can follow complex pathways, depending on various factors (Blouin et al., 2018). Romania's sandy soils form a special world in terms of flora and vegetation (Păun et al., 1983). Information on the spontaneous flora of these surfaces in Romania can be found in specialized works published since the beginning of the 20th century. Prodan (1925) is the first Romanian botanist to make the first inventory of vascular plants on sandy soils in Romania, identifying 548 taxa. Valuable information can also be found in the monumental work "Flora R.P.R.-R.S.R." (Săvulescu et al., 1952-1976) and in publications by botanists (Buia & Păun, 1958, 1964; Păun, 1967a, b; Păun & Popescu, 1972, 1983, Simeanu et al., 2019). The existence of sands in this part of the country should not be correlated with the presence of steppe (Buia, 1960). The areas occupied by sandy soils in Romania total about 370000 ha, i.e. about 1.6% of the country's surface (Mihalache & Ilie, 2008). A good part of these places are occupied by mobile and semi-mobile sands that have a poor water retention capacity. They are found in the western and south-western part of the Romanian Plain. In the literature they are known as "the sands of southern Oltenia" (Maxim et al., 1964). Changes, measuring the amplitude, direction, and rate of climate-induced changes in vegetation composition remain a serious challenge for specialists. The present work aimed to analyse the evolution of the floristic composition of an ecosystem in the sandy area of southwestern Romania under the influence of environmental factors.

MATERIALS AND METHODS

The study was carried out in the "museum plot", an area of land belonging to the Research-Development Station for Plant Culture on Sand, Dăbuleni (43°48′04″N 24°05′31″E). The area of land aims to preserve the spontaneous psammophilous vegetation of Oltenia. The total observation area comprises about 5 ha of unimproved sandy land, characterized by dunes and interdunes. From a climatic point of view, the area of sandy soils in the considered perimeter is classified, according to the Koppen classification, at the limit between Cf - temperate humid climate and BS - arid steppe climate (Șoimu et al., 2000; Dragomirescu, 1986). In the last ten years (2012-2021), the observation area includes mean annual temperatures ranging from 12.1°C -13.5°C and total annual precipitation ranging from 363 to 972.8 l/m^2. The wind pattern is characterized by a dominant V-E direction with a frequency of 59%. The strongest winds occur between March and June when intense deflation phenomena take place (Șoimu et al., 2000; Șoimu, 1998). Under the influence of these natural factors, the psammophilous vegetation has developed certain physiological adaptation characteristics. Wild flora and natural plant associations remained only on land that could not be cultivated for various reasons (Iuga & Stana, 2006). The first step in this work was to consult the bibliographic material on vascular psammophilous flora. Then, starting from this data, field trips were carried out several times in order to capture all the stages of development of the plants that would allow their correct identification. Where appropriate, specimens were also collected for those taxa where determination could not be made in the field. They were preserved by pressing, identified with the help of literature (Prodan, 1939; Beldie, 1977, 1979; Ciocârlan, 2000, 2009; Sârbu et al., 2013; Tutin et al., 1964-1980) and then included in the herbarium of the University of Craiova (C.R.A.). After the identification of all the material, a floristic list was compiled and compared with the one made about 40 years ago in order to observe the floristic dynamics of these sandy places and the influence of climatic changes on species distribution.

RESULTS AND DISCUSSIONS

The results obtained on the floristic composition of the research area, now and 40 years ago, are presented in Table 1. From the comparative analysis of the data inserted in Table 1, we found that there are some differences in floristic composition, which shows that climatic changes have influenced the physiognomy of these sites. Among the changes observed we mention: (1) reduction in the number of species from 157 to 130 taxa; (2) disappearance of some species (48 taxa) and appearance of others (26 taxa); (3) presence of new invasive species (*Ailanthus altissima*, *Gelditsia triacanthos*, *Phytolacca americana*, *Sorghum halepense*, *Morus alba*); (4) absence of wetland-loving plants (*Cyperus glomeratus*, *Juncus articulatus*, *Typha latifolia*, *Typha angustifolia*, *Trifolium fragiferum*, *Trifolium resupinatum*, etc), mentioned in studies by Păun et al. (1983), which explains the increasing aridity of these sites.

In terms of species reduction, according to studies by Yao et al. (2006) in arid and semi-arid regions, droughts can have major negative effects on plant species, but ground cover is spatially variable because species respond differently to different site conditions. Comparative analysis of the 1983/2022 moisture index (Figure 1) and the plant species present in the two periods analysed (Table 1) shows a decrease in the number of dryland-loving species, largely represented by annual plants growing in open areas (e.g. *Holosteum umbellatum*, *Ranunculus illyricus*, *Polycnemum majus*, *Polygonum arenarium*, etc.) and ruderal or segetal plants (e.g. *Solanum nigrum*, *Datura stramonium*, *Echium vulgare*, *Geranium pusillum*, *Lamium amplexicaule*, *L. purpureum*, etc). Yao et al. (2006) consider drought to be the primary factor shaping the structure and dynamics of arid and semi-arid grasslands. In terms of changing species structure, the comparative analysis of extinct and emerging species can be explained by the advanced uncultivated of these areas. Some of the extinct species have not been able to compete with those species that have formed a vegetation cover with a very good cover (about 90-95%). If we add to this the aggressiveness with which some newly emerged species have developed in the areas analysed and the invasive potential that they show more and more from one year to the next, it explains why in some taxa the populations have become very poor or even disappeared.

Among the invasive or potentially invasive species found in the surveyed territory, we mention both woody species (e.g. *Ailanthus altissima*, *Gleditsia triacanthos*, *Morus alba*) and herbaceous species (e.g. *Phytolacca americana*, *Sorghum halepense*). The invasive potential of the adventive species *Ailanthus altissima* is explained by rapid growth in the early years of vegetation, strong draining, and high drought resistance due to an extensive root system (Kowarik & Saumel, 2007; Udvardy 2008; Wittenberg, 2005). *Gleditsia triacanthos* is a tree with strong, branched thorns, which is why it has been cultivated as a hedge on these sandy soils. It has spread by winds that are quite frequent in this part of the country and by sprouts. It is a drought and frost-resistant plant (Dumitru-Tătăranu, 1960; Haralamb, 1967). *Morus alba* is drought resistant and relatively undemanding in soil fertility. Like the 'ash tree' it is fast-growing, sprouts very well, and is easily spread by birds, mammals, and man. *Phytolacca americana* is an unpretentious adventive species that does not require heat, likes light soils such as sands, and tolerates drought conditions quite well. Only late spring and early autumn frosts affect it. Park et al. (1998) reported that the growth of *Phytolacca americana* depends more on sunlight than on inorganic nutrients. *Sorghum halepense* is a rhizomatous species that where established multiplies predominantly vegetatively via rhizomes (Anghel et al., 1972). The high temperatures in sandy soils favor the germination of seeds of this plant which have multiple pathways of spread: wind, agricultural machinery, via animals, etc. *S. halepense* is well known for its detrimental effects on the growth and development of neighboring plants through its strong competition and allelopathic potential (Huang et al., 2015).

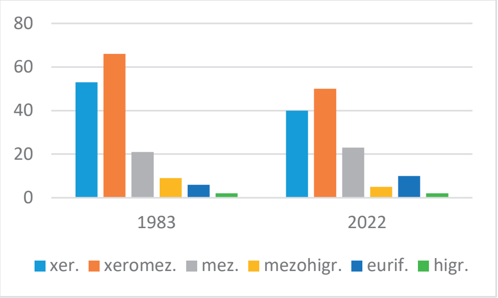

Figure 1. Humidity index analysis

The absence of some wetland-loving species that were mentioned about 40 years ago such as *Cyperus glomeratus*, *Juncus articulatus*, *Typha latifolia*, *Typha angustifolia*, *Trifolium fragiferum*, *Trifolium resupinatum* is explained by the destruction of irrigation systems that were used to irrigate crops in the past.

Table 1. List of species in the "museum plot"

Scientific name	Botanical family	Păun et al. 1983	Present 2022
Achillea ochroleuca Ehrh.; *A. setacea* Waldst. et Kit.	*Asteraceae*	+	+
Agrostis stolonifera L.	*Poaceae*	+	+
Ailanthus altissima (Mill.)	*Simaroubaceae*	-	+
Alkanna tinctoria Tausch.	*Boraginaceae*	+	-
Alyssum alyssoides (L.) L.; *A. desertorum* Stapf	*Brassicaceae*	+	+
Amaranthus albus L.; *A. retoflexus* L.	*Amaranthaceae*	+	+
Anagallis arvensis L.	*Primulaceae*	+	+
Anchusa officinalis L.	*Boraginaceae*	-	+
Anthemis arvensis L.	*Asteraceae*	-	+
Anthemis ruthenica M. Bieb.	*Asteraceae*	+	+
Apera spica-venti (L.) Beauv.	*Poaceae*	+	+

Scientific name	Botanical family	Păun et al. 1983	Present 2022
Arabidopsis thaliana (L.) Heynh.	*Brassicaceae*	+	+
Arenaria serpyllifolia L.	*Caryophyllaceae*	+	+
Aristolochia clematitis L.	*Aristolochiaceae*	+	-
Artemisia absinthium L.	*Asteraceae*	+	-
Bassia laniflora (S.G.Gmel.) A. J. Scott	*Chenopodiaceae*	+	+
Berteroa incana (L.) DC.	*Brassicaceae*	+	+
Bidens cernua L.; *B. tripartita* L.	*Asteraceae*	+	-
Bromus sterilis L.; *B. tectorum* L.	*Poaceae*	+	+
Capsella bursa-pastoris (L.) Medik.	*Brassicaceae*	+	+
Centaurea arenaria M. Bieb. subsp. *borysthenica* (Gruner) Dostál; *Centaurea calcitrapa* L., *Centaurea solstitialis* L. ssp. solstitialis	*Asteraceae*	+	+
Centaurea cyanus L.; *C. solstitialis* L.	*Asteraceae*	+	-
Cerastium dubium (Bast.) Guépin); *C. semidecandrum* L.	*Caryophyllaceae*	+	+
Cerinthe minor L.	*Boraginaceae*	+	-
Chamaecytisus austriacus (L.) Link	*Fabaceae*	+	+
Chenopodium album L.; *Ch. botrys* L.; *Ch. strictum* Roth	*Chenopodiaceae*	+	+
Chondrilla juncea L.	*Asteraceae*	+	-
Cichorium intybus L.	*Asteraceae*	+	-
Cirsium arvense (L.) Scop.; *C. vulgare* (Savi) Ten	*Asteraceae*	+	+
Clinopodium vulgare L. (*Calamintha vulgaris* (L.) Druce.)	*Lamiaceae*	+	-
Consolida regalis S. F. Gray	*Ranunculaceae*	+	-
Convolvulus arvensis L.	*Convolvulaceae*	-	+
Conyza canadensis (L.) Cronquist	*Asteraceae*	+	-
Crataegus monogyna Jacq.	*Rosaceae*	-	+
Crepis foetida L. subsp. *rhoeadifolia* (M. Bieb.) Čelak.	*Asteraceae*	+	+
Cruciata laevipes Opiz.	*Rubiaceae*	+	+
Cuscuta europaea L.	*Cuscutaceae*	+	+
Cynodon dactylon L.	*Poaceae*	+	+
Cyperus glomeratus L.	*Cyperaceae*	+	-
Datura stramonium L.	*Solanaceae*	+	-
Daucus carota L.	*Apiaceae*	+	+
Descurainia sophia (L.) Webb ex Prantl	*Brassicaceae*	+	+
Dichanthium ischaemum (L.) Roberty	*Poaceae*	+	+
Digitaria sanguinalis (L.) Scop.	*Poaceae*	+	+
Elymus repens)L.) Gould	*Poaceae*	+	+
Echinochloa crus-galli (L.) Beauv.	*Poaceae*	+	+
Echium vulgare L.	*Boraginaceae*	+	-
Equisetum arvense L.; *E. ramosissium* (Desf.)	*Equisetaceae*	-	+
Eragostis pilosa (L.) Beauv.; *E. minor* Host	*Poaceae*	+	+
Erodium cicutarium (L.) L'Hérit.	*Geraniaceae*	+	+
Erodium hoefftianum C. A. Mey. subsp. *neilreichii* (Janka) Soó	*Geraniaceae*	-	+
Erophila verna (L.) Chevall.	*Brassicaceae*	+	+
Eryngium campestre L.	*Apiaceae*	+	+
Erysimum diffusum Ehrh.	*Brassicaceae*	+	-
Euphorbia seguieriana Neck.; *Euphorbia virgata* Waldst. et Kit.	*Euphorbiaceae*	+	+
Falcaria vulgaris Bernh.	*Apiaceae*	+	-
Fallopia convolvulus (L.) Á. Löve	*Polygonaceae*	+	+
Festuca valesiaca Schleich. ex Gaudin	*Poaceae*	+	+
Filago arvensis L.	*Asteraceae*	+	+
Fumaria schleicheri Soy.-Willem.	*Papaveraceae*	+	-
Gagea villosa (M. Bieb.) Sweet	*Liliaceae*	+	+
Gagea pusilla (F. W. Schmidt). Schult. et Schult. f.	*Liliaceae*	+	+
Galinsoga parviflora Cav.	*Asteraceae*	+	-
Galium aparine L.	*Rubiaceae*	+	+
Geranium pusillum Burm.	*Geraniaceae*	+	-
Gleditsia triachanthos (L.)	*Fabaceae*	-	+
Gypsophila muralis L.	*Caryophyllaceae*	+	-
Heliotropium europaeum L.	*Boraginaceae*	+	-
Hieracium pilosella L.	*Asteraceae*	+	+
Holosteum umbellatum L.	*Caryophyllaceae*	+	-
Hordeum murinum L.	*Poaceae*	+	+
Hyoscyamus niger L.	*Solanaceae*	+	-
Hypericum elegans Stephan; *Hypericum perforatum* L.	*Hypericaceae*	+	+
Jasione heldreichii Boiss. & Orph.	*Campanulaceae*	+	+
Juncus articulatus L. em Richt.	*Juncaceae*	+	-

Scientific name	Botanical family	Păun et al. 1983	Present 2022
Kohlrauschia prolifera (L.) Kunth	*Caryophyllaceae*	+	+
Lactuca serriola L.	*Asteraceae*	+	+
Lamium amplexicaule L.; *Lamium purpureum* L.	*Lamiaceae*	+	-
Leonorus cardiaca L.	*Lamiaceae*	+	-
Linaria genistifolia (L.) Mill.	*Scrophulariaceae*	+	+
Linaria vulgaris Mill.	*Scrophulariaceae*	+	-
Lithospermum arvense L.	*Boraginaceae*	+	+
Lotus corniculatus L.	*Fabaceae*	+	+
Malva sylvestris L.	*Malvaceae*	-	+
Marrubium peregrinum L.	*Lamiaceae*	+	+
Marrubium vulgare L.	*Lamiaceae*	+	-
Medicago falcata L.; *Medicago lupulina* L.; *Medicago minima* (L.) L.	*Fabaceae*	+	+
Melica ciliata L.	*Poaceae*	-	+
Mollugo cerviana (L.) Ser.	*Molluginaceae*	+	+
Morus alba L.	*Moraceae*	-	+
Muscari neglectum Guss. ex Ten.	*Liliaceae*	+	-
Onopordum acanthium L.	*Asteraceae*	+	+
Onosma heterophylla Griseb.	*Boraginaceae*	+	-
Ornithogalum umbellatum L.	*Liliaceae*	+	+
Orobanche arenaria Borkh.	*Orobanchaceae*	+	-
Papaver dubium (L.) var. *albiflorum* Elk.	*Papaveraceae*	-	+
Papaver rhoeas L.	*Papaveraceae*	+	-
Phragmites australis (Cav.) Steud.	*Poaceae*	-	+
Phytolacca americana L.	*Phytolaccaceae*	-	+
Plantago arenaria Waldst. & Kit. (*P. indica* L.)	*Plantaginaceae*	+	+
Poa bulbosa L. subsp. *vivipara* (Koeler) Arcang.	*Poaceae*	+	+
Poa pratensis L.; *P. trivialis* L.	*Poaceae*	-	+
Polycnemum majus A. Br.	*Chenopodiaceae*	+	-
Polygonum arenarium Waldst. et Kit.; *P. aviculare* L.	*Polygonaceae*	+	-
Portulaca oleracea L.	*Portulacaceae*	+	-
Potentilla argentea L.; *P. reptans* L.	*Rosaceae*	+	+
Prunus cerasifera Ehrh.	*Rosaceae*	-	+
Padus avium Mill.	*Rosaceae*	-	+
Ranunculus illyricus L.	*Ranunculaceae*	+	-
Robinia pseudoacacia L.	*Fabaceae*	+	+
Rosa canina L.	*Rosaceae*	-	+
Rumex acetosella L.	*Polygonaceae*	+	-
Salix alba L.	*Salicaceae*	-	+
Salsola kali L. subsp. *ruthenica* (Iljin.) Soó	*Chenopodiaceae*	+	+
Sambucus ebulus L.	*Caprifoliaceae*	+	-
Scabiosa argentea L. (*S. ucranica* L.)	*Dipsacaceae*	+	+
Scleranthum polycarpos L.	*Caryophyllaceae*	+	-
Senecio vernalis Waldst. et Kit.	*Asteraceae*	+	+
Setaria pumila (Poir.) Roem. et Schult.; *S. verticillata* (L.) Beauv.; *S. viridis* (L.) Beauv.	*Poaceae*	+	+
Silene conica L.; *S. borystenica* (Gruner) Walters; *S. latifolia* Poir. subsp. *alba* (Mill.) Greuter et Burdet; *S. vulgaris* (Moench) Garcke	*Caryophyllaceae*	+	+
Sisymbrium orientale L.	*Brassicaceae*	+	-
Solanum nigrum L.	*Solanaceae*	+	-
Sonchus arvensis L.	*Asteraceae*	+	+
Sorghum halapense (L.) Pers.	*Poaceae*	-	+
Stellaria media (L.) Vill.	*Caryophyllaceae*	+	+
Syrenia cana (Pill. et Mitterp.) Neilr.	*Brassicaceae*	+	+
Thlaspi arvense L.; *Th. perfoliatum* L.	*Brassicaceae*	+	+
Torilis arvensis (Huds.) Link	*Apiaceae*	+	+
Tragopogon floccosus Waldst. et Kit.	*Asteraceae*	+	+
Tragus racemosum (L.) All.	*Poaceae*	+	+
Tribulus terrestris L.	*Zygophyllaceae*	+	+
Trifolium arvense L.; *T. campestre* Schreb.; *Trifolium echinatum* M. Bieb.; *T. pratense* L.; *T. repens* L.	*Fabaceae*	+	+
Trifolium fragiferum L.; *T. retusum* L.; *T. resupinatum* L.	*Fabaceae*	+	-
Typha angustifolia L.; *T. latifolia* L.	*Typhaceae*	+	-
Verbascum phlomoides L.; *V. thapsus* L.	*Scrophulariaceae*	+	+

Scientific name	Botanical family	Păun et al. 1983	Present 2022
Verbena officinalis L.	*Verbenaceae*	+	-
Veronica acinifolia L.; *V. hederifolia* L. subsp. *triloba* (Opiz) Čelak.; *V. triphyllos* L.	*Scrophulariaceae*	+	+
Vicia villosa Roth.	*Fabaceae*	-	+
Viola kitaibeliana Schult.	*Violaceae*	+	+
Vulpia myuros (L.) C.C. Gmel.	*Poaceae*	+	+
Xanthium italicum Moretti; *X. spinosum* L.; *X. strumarium* L.	*Asteraceae*	+	+
Xeranthemum annuum L.	*Asteraceae*	-	+

[1](+) = the presence of the species;
[2](-) = the absence of the species.

CONCLUSIONS

In conclusion, in areas occupied by sandy soils where the influence of the anthropic factor is reduced to a minimum, a greater cover of vegetation built by species that are not characteristic of sands, but which grow and develop relatively well on sands (e.g. *Cynodon dactylon*, *Bromus tectorum*, *Digitaria sanguinalis*, etc.) is observed. Annual plants which in the past formed self-sustaining associations (e.g. *Mollugetum cervianae* Borza 1961) are now rare in the few remaining sandy meshes not covered by vegetation. Although seeds or clones of typical psammophilous species (*Festuca vaginata* Waldst. et Kit., *Koeleria glauca* (Schkuhr) DC., *Secale sylvestre* Host., *Trigonella monspeliaca* L., *Teucrium polium* L., *Helichrysum arenarium* (L.) Moench) were brought to the reserve about 40 years ago, they have not adapted to these sites. Typical psammophilous species such as *Polygonum arenarium, Mollugo cerviana, Silene conica, Tragus racemosus, Plantago arenaria*, *Jasione heldreichii, Achillea ochroleuca, Centaurea solstitialis,* etc., prefer open sites because they do not seem to tolerate competition with other species.

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EVALUATION OF THE ORGANOLEPTIC CHARACTERISTICS AND PHENOLIC COMPOUNDS OF AROMATIC/MEDICINAL PLANTS FROM URBAN HORTICULTURE

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Abstract

Cultivation of aromatic/medicinal plants (MAPs) is major part of urban horticulture and landscape architecture, several of MAPs also being widespread in the medical industry due to their nutrient content. In this work, an organoleptic evaluation of infusions and condiments from 25 MAPs was carried out, with the participation of 45 different tasters. The bioactive compounds in the infusion of some MAPs were then evaluated. The selected plants were grown in a greenhouse, through urban co-cultivation, in Bucharest and Thessaloniki. The appearance, smell, taste and aroma were assessed from the organoleptic characteristics, and the obtained results were processed with the SPSS statistical program. The DPPH and Folin-Ciocalteu methods were used to measure antioxidant and total phenolic concentration. The purpose of the work is to examine the relationship between the organoleptic characteristics and the nutritional elements of some MAPs. Also, it is sought to form an objective image regarding the role and the way of the use of MAPs so that they become acceptable to consumers, in their daily diet. The results confirm that the co-cultivation of some MAPs is an activity with significant prospects in urban horticulture and healthy nutrition.

Key words: *Aromatic/medicinal plants, organoleptic evaluation, bioactive compounds, urban horticulture.*

INTRODUCTION

It is an undisputed fact that MAPs play an important role in human well-being, while their cultivation in the urban fabric also contributes to the goals of sustainable urban horticulture (Maknea et al., 2023). The analysis of medicinal plants has a long history in assessing the quality of a plant and the first analyses performed were organoleptic (Fitzgerald et al., 2020). Aromatic/medicinal plants have been used since ancient times for various purposes, such as treating infectious diseases, producing perfumes, preserving food (protection against pathogenic micro-organisms and deterioration, protection of organoleptic characteristics), etc. (Sakkas & Papadopoulou, 2017). However, in terms of their use, plants are more appreciated in the food sector than in the pharmaceutical sector (de Medeiros et al., 2021).

Today, there is also growing interest in MAPs as natural alternatives to synthetic preservatives in food. This is because herbs are an excellent source of antioxidants and antimicrobial's (Nieto, 2020) and because of their role in the Mediterranean diet. This is confirmed and reinforced by the fact that in 2010 the Mediterranean Diet was included in UNESCO's Representative List of the Intangible Cultural Heritage of Humanity. The aim is to safeguard the cultural value of the Mediterranean Diet and to share and disseminate its values and benefits, internationally (Serra-Majem et al., 2012). It has also been noted that the use of MAPs has increased in some geographical areas during COVID-19, due to the characteristics of MAPs (Khadka et al., 2020).

However, little research has been carried out on the effect of the microenvironment on plant adaptation, phenolic composition and sensory control (Kabtni et al., 2020). This control includes the evaluation of appearance, smell, taste, and aroma performed by the human sensory system, which enables the perception of taste and smell and transmits information about sensory properties (Renna et al., 2017).

In the case of MAPs, the most common form of consumption is tea. Its consumption is influenced by many factors, especially those related to cultivation, health and sensory properties (Rocha et al., 2020). In general, consumer acceptance and the development of sustainable, healthy and tasty products are closely linked (Pointke et al., 2022).

This paper presents a multifaceted approach to 25 species of medicinal aromatic plants (MAPs), grown in covered areas in different cities. In order to give consumers a wide choice of plants to grow and consume, it was decided to showcase many plants. These MAPs can be grown together or with vegetables in pots, urban gardens, etc. (urban horticulture), contributing both nutritionally and ornamentally through their aromatic and medicinal properties. They also contribute to the protection of beneficial insects such as bees in urban areas where environmental conditions permit their cultivation. Furthermore, urban horticulture is an alternative strategy for sustainable development and urban enhancement (Maknea et al., 2022). In this context, some questions are raised, which are the purpose of this paper. Specifically, what are the benefits to the customer of finding the plant attractive from an organoleptic point of view, and how? Also, whether their antioxidant properties change depending on the form of consumption and in which form the properties are retained, more.

The proposed research goals have been achieved through two separate phases (organoleptic and qualitative evaluation). Firstly, consumer preference was tested in terms of organoleptic (sensory) characteristics of the MAPs. These characteristics reflect the specific profile of a plant. This was followed by analysis of specific quality parameters such as polyphenols and antioxidant capacity in 15 samples of infusions.

MATERIALS AND METHODS

Plant materials

For this research, 15 MAPs cultivated in Romania, including 13 in Bucharest-B and 2 in Buzău-Bz, and 10 MAPs cultivated in Greece (Thessaloniki-SKG) were chosen, as shown in Table 1. For growing the MAPs (apart from the plants grown in Buzău), plant material derived from traditional Greek varieties was used, which is part of a research monitoring their adaptation to climate change in the Balkan countries. The 25 samples used in the event were leaves of MAPs, collected and dried in rooms with controlled heating (22°C).

Table 1. Description of the material used for organoleptic evaluation

Code	Plants	Family	City	Food group
1	*Aloysia citrodora*	Verbenaceae	B	1
2	*Aloysia citrodora*	Verbenaceae	SKG	1
3	*Origanum dictamnus*	Lamiaceae	SKG	1
4	*Syderitis syriaca-malotira*	Lamiaceae	B	1
5	*Syderitis syriaca-malotira*	Lamiaceae	SKG	1
6	*Sideritis scardica-Domnesc*	Lamiaceae	BZ	1
7	*Mentha spicata*	Lamiaceae	B	1
8	*Mentha piperita*	Lamiaceae	B	1
9	*Origanum majorana*	Lamiaceae	B	1
10	*Origanum majorana*	Lamiaceae	SKG	1
11	*Rosmarinus officinalis*	Lamiaceae	B	1
12	*Salvia officinalis*	Lamiaceae	B	1
13	*Lophantus anisatus*	Lamiaceae	BZ	1
14	*Origanum vulgare*	Lamiaceae	B	2
15	*Origanum onites*	Lamiaceae	SKG	2
16	*Satureja thymbra*	Lamiaceae	B	2
17	*Satureja thymbra*	Lamiaceae	SKG	2
18	*Origanum majorana*	Lamiaceae	B	3
19	*Origanum majorana*	Lamiaceae	SKG	3
20	*Satureja thymbra*	Lamiaceae	B	3
21	*Satureja thymbra*	Lamiaceae	SKG	3
22	*Thymus vulgaris*	Lamiaceae	B	4
23	*Thymus citrodorus*	Lamiaceae	B	4
24	*Thymus citrodorus*	Lamiaceae	SKG	4
25	*Crithmum maritimum*	Apiaceae	SKG	5

Sensorial evaluation

For the organoleptic evaluation, a free tasting event was organized at the USAMV Bucharest premises to evaluate the selected MAPs and to promote the Mediterranean diet. For this, we selected several MAPs that are most commonly used in Mediterranean cuisine. Afterwards, they were presented and offered in different forms and ways (tasting proposals) with criteria, their role in Mediterranean cuisine, creating 5 food groups (1=tea, 2=cheese & olive oil, 3=focaccia & olive oil, 4=olives & olive oil, 5=pickles) according to Table 1.

During the event, leaflets with information on the characteristics and consumption patterns of MAPs were distributed. Tasting suggestions (samples) were presented at stands. These were grouped by food group and homogenized in terms of container shape, quantity, and temperature.

Questionnaires were then distributed to collect information on consumers' acceptance of the food samples and their perception of sensory attributes.

The descriptive sensory evaluation was carried out using a group of testers of different ages and not experts. This was done to provide an objective basis, as reported in a related study by Drake (2022). The questionnaires were divided into five sections according to the food group to which each sample was assigned. For the evaluation, participants were asked to rate the smell, taste, appearance and aroma of each of the 25 samples using a structured 5-point Likert scale, with 1 indicating "I don't like it very much" and 5 "I like it very much" (Bhandari & Nikolopoulou, 2023). 45 questionnaires were answered for the processing of which statistical software packages (IBM SPSS Statistics and Microsoft Excel) were used. Questionnaires were grouped in 4 age groups (20-30, 30-45, 45-65, over 65) (Table 2). For each age group, descriptive statistical analysis (mean number, standard deviation) and frequency distribution (frequency, percent, valid percent, cumulative percent) were performed.

Physicochemical evaluation

Dry matter content

The dry matter (DM) content was determined using the gravimetric method - removing water by evaporation and weighing - and the results were expressed as percentages. In particular, the total dry matter was determined by weighing 1 g of the sample and then drying it at 105°C with the aid of a MAC 50 PARTNER thermobalance (Badea et al., 2022).

Bioactive compound extraction

To assess the total polyphenol content and the antioxidant activity of the MAPs, two extraction methods have been used on 15 samples of MAPs. The first method was water hot extraction (infusion) of the MAPs dried samples using specific mass, volume, and extraction time (Table 4). Immediately after the extraction was completed, the sample extracts were cooled on an ice bath and subjected to analysis. For the second extraction, a 70% methanol solution was used. Approximately 0.2 g of the material is weighed, and 30 mL of a 70% methanol solution was added. The extraction procedure was similar to that described by Ion et al. (2020).

Determination of total polyphenols content

To quantify the total polyphenols content, the Folin-Ciocalteu method was used, according to a protocol adapted from Stan et al. (2021). Briefly, 2.5 mL of Folin-Ciocalteu reagent (the reagent was diluted 10 times) and 2 mL of 7.5% sodium carbonate were added to 500 μL of the sample. It was incubated for 15 minutes at 50 C in a water bath, allowed to cool on an ice bath and read at a wavelength of 760 nm.

DPPH method for the determination of antioxidant activity

To measure the antioxidant activity, 200 μL of the extracts solution were mixed with 2 mL of DPPH solution (0.2 M) in methanol. The mixture was agitated for 30 minutes in the dark by magnetic shaking. After the incubation, the absorbance was measured at a wavelength of 515 nm (Ion et al., 2020).

Statistical analysis

All results obtained from measuring both types of samples were processed using MS EXCEL and Open Document.

RESULTS AND DISCUSSIONS

The data obtained from the study and from processing the results of the questionnaires and qualitative analyses are presented in tables and graphs.

MAPs organoleptic evaluation

For the sensory analysis, the grouping of participants by age was chosen because it is a criterion for objective assessment of smell, taste and vision, and because it is known that the sensory sensitivity of older people varies as they lose part of their sense of smell and taste over time (Cain & Steven, 1989). Therefore, the statistical analysis was based on 4 variables: number of completed questionnaires, age of testers, samples (taste suggestions) and scores.

The 45 completed questionnaires collected are considered a satisfactory number (Caracciolo et al., 2020), which reinforces the objectivity of the conclusions. In terms of age, of the 45 participants, 35.5% were aged 20-30, 26.6% were aged 30-45, 31.3% were aged 45-65 and 6.6% were over 65 (Table 4). The general results of the sensory evaluation show (Figure 1) that the criterion "appearance" received the highest score (5) with 52.3% of the respondents, while "taste" and "aroma" received the lowest score (1) with 2.3% of the respondents.

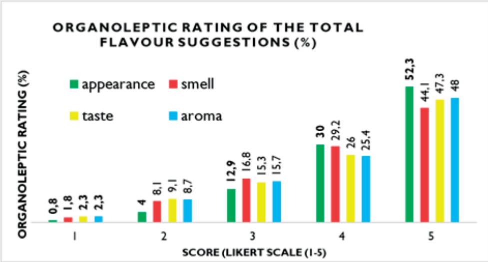

Figure 1. Total sensory evaluation of samples by rating scale

To continue the statistical treatment of the data, it was considered that the results should be grouped by sensory characteristic. Therefore, Figure 2 shows the complete "appearance" rating of the flavor proposals based on the MAPs. As can be seen, the highest preference was expressed for sample (18) with 70.3%, while sample (9) received the lowest preference (5.3%).

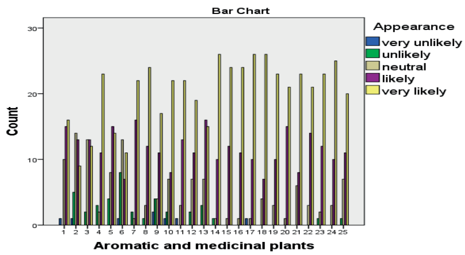

Figure 2. Evaluation of the "appearance" of the samples

Similarly, the visualization of the evaluation of "smell" (Figure 3) showed that sample (8) received the highest score with 65%, while sample (9) received the lowest score with 7.9%. In the evaluation of "taste" (Figure 4), sample (9) received the highest percentage (12.8%) with the lowest score, while only 7.4% of the testers rated sample (14) as excellent. Finally, in Figure 5, sample (9) received the lowest score for "aroma" with 10.5% and the highest for sample (15) with 75.7%.

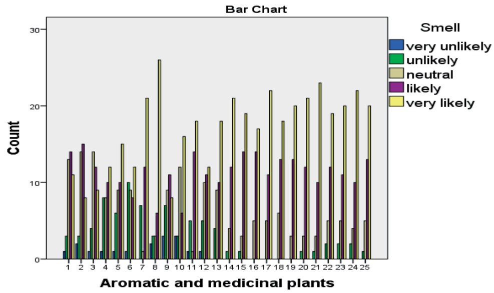

Figure 3. Evaluation of the "smell" of the samples

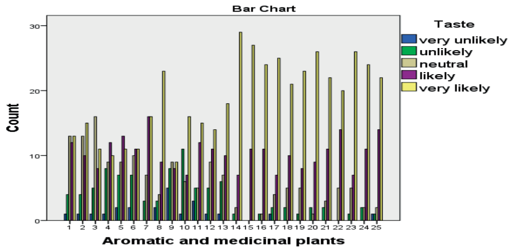

Figure 4. Evaluation of the "taste" of the samples

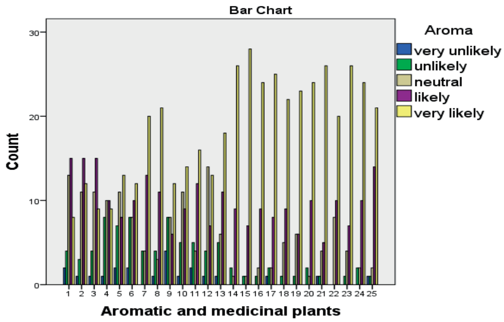

Figure 5. Evaluation of the "aroma" of the samples

As a continuation of the study of the response of the testers to the characteristics of the sample, the criterion of age was considered as a key factor in the evaluation. It was therefore decided to distribute the results of the statistical analysis by age group (Table 2) and to interpret them in terms of maximum and minimum scores. Considering the very low participation rate in the 65+ age group, it was decided to ignore some of the scoring data. The table shows that a very high percentage (84.9%) of the sample (18) were visually assessed by the testers in the 20-30 age group. At the same time, they rated the sample (9) negatively in terms of smell, taste and aroma. Or the age group (30-45), the highest percentage (90%)

with the highest rating gave the sample (8) for "smell". Finally, the results show that 92.3% of the age group (45-65) preferred sample (16) for taste and sample (15) for aroma, while 8.3% did not like the appearance of sample (9) at all. Note that the bold values in Table 2 indicate the samples with the highest sensory preference.

Table 2. Record evaluation results of tasting proposals with minimum (1) and maximum (5) scores

Age group / percent tasters	Scores	Appearance		Smell		Taste		Aroma	
	Min / Max	% of tasters	Samples(code)	% of tasters	Samples(code)	% of tasters	Samples(code)	% of tasters	Samples (code)
All testers	1	5.3	9	7.9	9	12.8	9	10.5	9
	5	**70.3**	14;17; 18	**65.0**	8	**74.4**	14	**75.7**	15
20-30 35.5%	1	7.1	9;10;11	14.3	9.10	28.6	9	21.4	9
	5	**84.6**	18	60.0	8	73.3	14	**84.6**	21
30-45 26.6%	1	**8.3**	1;2	8.3	1;2	8.3	1;2	8.3	1;2
	5	80.0	4	**90.0**	8	75.0	14.15	70.0	23
45-65 31.3%	1	**8.3**	9	8.3	9	8.3	9	8,3	9
	5	75.0	21	76.9	16;24	**92.3**	16	**92.3**	15
Up to 65 6.6%	1	50.0	4;6;7;8;9;10	50.0	4;6;7;9;12	33.3	4;6;7;9;12;13	50.0	4;6;7;9;1 2;13
	5	100.0	17	100.0	17	100.0	24;25	100.0	25

The results show that *Origanum majorana* (RO), presented with bread and olive oil, is among the samples with the highest preference, both in the age group (20-30 years) and in all tasters, based on visual inspection. On the other hand, the same MAP, when presented in the form of tea, was the least preferred. This shows that the way the food is used and presented is an important criterion for its selection. This conclusion is supported by the fact that the characteristic "appearance" was rated as the most important criterion in the sensory analysis, followed by aroma, taste and appearance. Moreover, it is documented in the literature that the appearance of food products strongly influences consumer choice (Caracciolo et al., 2020). It should be noted that this herb performed quite well in the other sensory tests. However, it didn't perform very well in the qualitative analysis scoreboard (dry sample), based on antioxidant capacity and total phenolics.

Another interesting point is the fact that there are common preferences between the groups (20-30) and (30-45), which makes it possible to highlight some proposals. There is a joint evaluation with the maximum degree of *Mentha piperita* (RO) for its smell in tea and *Origanum vulgare* (RO) for its taste combined with cheese and olive oil. This result is confirmed by data from the grey literature, where analyses of the sample "cheese with *Origanum vulgare* extract 6%" showed very high levels of nutrients (Kallipoliti & Christodoulou, 2021).

The age group (45-65) preferred *Satureja thumbra* (RO) with bread and olive oil based on taste and smell. In general, different results per age group confirm that there is a variation in sensory sensitivity of people of different ages, as the senses change over time (Cain & Steven, 1989).

Furthermore, the fact that tea from *Aloysia citrodora* received the highest percentage of negative ratings for all sensory characteristics by the group (30-45) does not make it popular with the Romanian public. A review of the grey literature reinforces the view that a similar organoleptic study in Mediterranean countries would yield different results. Moreover, cultural criteria influence sensory evaluation (Pearcey& Zhan, 2018).

MAPs qualitative evaluation

The application of the Folin-Ciocatleu method determined the total phenolic content and the application of the DPPH method resulted in the antioxidant capacity values of the dried leaf samples and the MAPs infusion samples. Specifically, Table 3 presents the results of qualitative analyses of 15 samples of dried MAPs leaves, observing that the dried leaves of *Sideritis syriaca-malotira* (Romania) contain the highest antioxidant components (1025.60±7.61 mg TE/g) and total phenolics (80.56 ±1.25 mg GAE/g), while *Mentha piperita* (Romania) has the lowest antioxidant capacity (364.49±24.32 mg TE/g) and the least total phenolics (26.81±0.31 mg GAE/g). Table 4 shows the results of the qualitative analyses of 14 infusions of dried leaves of MAPs, due to the insufficient number of samples for *Origanum majorana* (Romania). Among them, the tea with *Thymus citrodorus* (Romania) showed the highest value in antioxidant capacity (1273.71±11.26 mg TE/200 mL tea) and total phenolics (72.93±2.67 mg GAE/ 200 mL tea), while the lowest value for antioxidant components (23.52±2.79 mg TE/200 mL tea) and total polyphenols (2.54±0.09 mg GAE/200 mL tea) was found in *Sideritis syriaca-malotira* (Greece).

Table 3. Chemical composition of died plant samples

Code	Name of medicinal/aromatic plants	Cultivation Country	SU %	CTP (mg GAE/g)	AA (mg TE/ g)
1	*Aloysia citrodora*	Romania	92.34 ± 0.32	57.49 ± 1.48	757.58 ± 23.62
2	*Thymus vulgaris*	Romania	92.53 ± 0.68	63.52 ± 2.87	693.55 ± 30.53
3	*Thymus citrodorus*	Romania	92.76 ± 0.16	70.13 ± 1.03	936.12 ± 7.99
4	*Salvia officinalis*	Romania	91.12 ± 0.08	61.61 ± 10.00	876.32 ± 5.83
5	*Mentha spicata*	Romania	90.75 ± 0.57	38.83 ± 1.08	469.40 ± 33.61
6	*Rosmarinus officinalis*	Romania	91.83 ± 0.45	69.30 ± 1.08	932.04 ± 27.44
7	*Mentha piperita*	Romania	91.36 ± 0.55	26.81 ± 0.31	364.49 ± 24.32
8	*Origanum vulgare*	Romania	91.07 ± 0.28	62.52 ± 2.86	692.01 ± 9.48
9	*Sideritis syriaca-malotira*	Romania	93.83 ± 0.25	80.56 ± 1.25	1025.60 ± 7.61
10	*Satureja thymbra*	Romania	93.23 ± 0.12	66.15 ± 5.17	820.55 ± 90.40
11	*Origanum majorana*	Romania	93.25 ± 0.30	53.64 ± 1.33	545.35 ± 16.22
12	*Satureja thymbra*	Greece	91.17 ± 0.13	72.78 ± 1.15	923.78 ± 41.39
13	*Origanum onites*	Greece	91.52 ± 0.29	60.41 ± 1.94	604.40 ± 19.79
14	*Sideritis syriaca-malotira*	Greece	93.33 ± 0.16	34.75 ± 0.46	411.46 ± 17.23
15	*Aloysia citrodora*	Greece	93.03 ± 0.08	54.73 ± 1.08	897.88 ± 33.81

Table 4. Chemical composition of tea samples

Code	Name of medicinal/aromatic plants	Cultivation	Plant weight (g)	V of water (mL)	Tea extraction time (min)	CTP (mg GAE/ 200 mL tea)	AA (mg TE/ 200 mL tea)
1	*Aloysia citrodora*	Romania	0.5	200	10	12.28 ± 0.15	193.38 ± 10.58
2	*Thymus vulgaris*	Romania	1	200	10	36.14 ± 2.58	533.81 ± 46.10
3	*Thymus citrodorus*	Romania	2	200	10	72.93 ± 2.67	1273.71 ± 11.26
4	*Salvia officinalis*	Romania	1.35	200	10	24.21 ± 1.02	347.84 ± 16.42
5	*Mentha spicata*	Romania	1.5	200	10	7.74 ±1.68	130.73 ± 29.11
6	*Rosmarinus officinalis*	Romania	1.4	200	20	6.91 ± 0.27	93.82 ± 4.75
7	*Mentha piperita*	Romania	1.5	200	10	18.35 ± 1.75	293.13 ± 17.68
8	*Origanum vulgare*	Romania	1.5	200	10	35.43 ± 1.53	521.86 ± 21.49
9	*Sideritis syriaca-malotira*	Romania	1	200	10	7.56 ± 1.41	111.95 ± 3.38
10	*Satureja thymbra*	Romania	0.8	200	10	33.09 ± 1.06	527.88 ± 59.80
11	*Origanum majorana*	Romania	-	-	-	-	-
12	*Satureja thymbra*	Greece	0.8	200	10	10.21 ± 0.32	121.13 ± 22.75
13	*Origanum onites*	Greece	0.7	200	10	12.88 ± 0.51	136.21 ± 10.29
14	*Sideritis syriaca-malotira*	Greece	1	200	10	2.54 ± 0.09	23.52 ± 2.79
15	*Aloysia citrodora*	Greece	0.5	200	10	3.75 ± 0.30	33.13 ± 3.34

Figure 6 shows the relationship between DPPH and TPC measurements in the dried MAPs leaf samples before and after their use as a decoction. Specifically, the data from Tables 3 & 4 have been used in the graph, in percentages, with the positive axis (+Y) chosen for the distribution of the percentages of nutrient reduction of MAPs from the spice form to the tea form. As can be seen, in the sample (3) the percentage change is -36.06% for DPPH and -3.99% for TPC. For the other samples, the rate of change is between (+19.58% - +96.31%) for the DPPH and (+31.54% - +93.15%) for the TPCs.

In order to assess the suitability and reliability of the methods used, linear regression and correlation analyses were carried out on the values, total antioxidant capacity and total phenolics, of the dried leaf samples of MAPs (Figure 8) and their infusion samples (Figure 9). The correlation of the percentages (Figure 6) representing the changes in the values of DPPH and TPC in the dry and wet samples is evaluated in Figure 7.

The coefficients of correlation (r) and coefficients of determination (r^2) were calculated using Microsoft Excel 2000. All (r) values are positive (r/tea = 0.99, r/dried plant = 0.89, r/change = 0.99), as are the (r^2) values (r^2/tea = 0.99 and r^2/dried plant = 0.80, r^2/change = 0.99). Therefore, we can easily conclude that the positive values and the linear correlation demonstrate, firstly, the relationship between total phenolics and antioxidant capacity and, secondly, the fact that phenolic compounds contribute significantly to the antioxidant properties of the selected plants (Tusevski et al., 2014).

The MAPs, *Thymus citrodorus* (RO), *Thymus vulgaris* (RO), *Origanum vulgare* (RO), used in the form of tea (infusion), appeared in the first 3 positions with the highest values of total phenolics and antioxidant capacity. In spice form (dried leaves), the highest bioactive compounds were measured in the samples of *Sideritis syriaca-malotira* (RO), *Satureja thumbra* (GR), *Thymus citrodorus* (RO) and *Rosmarinus officinalis* (RO). Although *Origanum vulgare* (RO), when used as a spice, received the highest score for taste, it did not rank among the best when tested for quality. It is worth mentioning that in studies with dry leaf samples, *Origanum vulgare* (from the region of Northern Macedonia) had twice as much phenolics (Tusevski et al., 2014), while the same plant from the region of Turkey (Kırca & Arslan, 2008) had 30% more than the sample of the present study.

Meanwhile, a study published in 2020 confirmed the significant contribution of *Rosmarinus officialis* to human health as one of the spices with the highest antioxidant content and antibacterial and anticancer activity (Kloy et al., 2020).

An academic study of MAP from the Pilion (Greece) found that the native *Thymus citrodorus*, followed by *Mentha spicata*, had the highest total phenolic content, with values of 56.33 and 33.53 mg GAE/g (Vengopoulos, 2020), while the corresponding values for the samples of this study are higher (70.13 and 38.83 mg GAE/g).

Our results are also consistent with those obtained in a study carried out with herbal infusions, which they wanted to highlight because they are considered one of the most consumed beverages in the world and are rich in polyphenolic compounds. In particular, the ratio between the antioxidant capacity values and the total phenolic content of *Mentha piperita* and *Aloysia citrodora* from the region of Argentina (Rodríguez et al., 2010) is the same as the corresponding ratio of the tea samples in this study, with different absolute values.

From the figures and tables of results, conclusions can be drawn for further research, as for example for *Aloysia citrodora*, which, according to the results, seems to be influenced, in addition to the environmental conditions, by the way in which it is cultivated. This can be deduced from the fact that the same herb cultivated in Thessaloniki has a lower antioxidant capacity and a lower total phenolic value than the corresponding one cultivated in Bucharest. Therefore, the method of cultivation (in rows or in a potting arrangement) is another topic for further research.

It was also observed that in a sample to which a combination of extracts of different MAPs was added, the antioxidant capacity was significantly higher than in the sample to which an extract of a single MAP was added (Kallipoliti & Christodoulou, 2021).

Another approach to the results of this study concerns the relationship between sensory and qualitative characteristics. For example, based on the results of the organoleptic analysis, *Origanum vulgare* (RO) received the highest percentage of positive taste ratings in tea form, while in the qualitative analysis, it was among the top MAPs with the highest total phenolics and antioxidants.

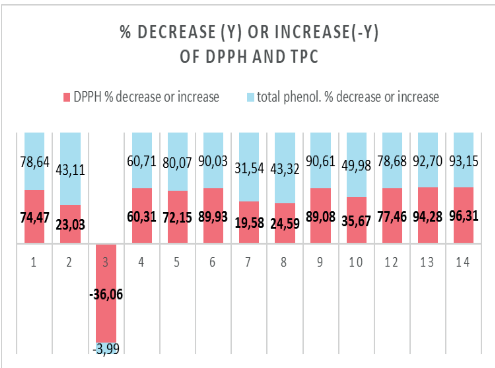

Figure 6. Change in AA & TPC values

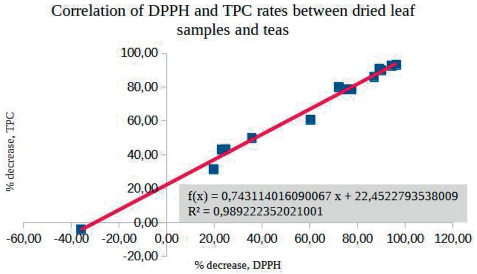

Figure 7. Correlation of AA & TPC

Many factors could explain the difference between the results of this study and those in the literature, such as the growing conditions of MAPs, the season and method of harvesting (Lemos et al., 2017), and even the way the leaves are dried. This is an issue that has been of interest to the scientific community, and the result of a study, mentions the fact that fresh *Origanum vulgare* has a higher content of total phenolics than the dried plant. It is also reported that the corresponding commercial samples have lower prices of bioactive ingredients than the dried ones grown in a personal space. In addition, using different Lamiaceae herbs has been shown to increase antioxidant and antibacterial activity (Chan et al., 2012).
It seems that Rababah et al. (2015) considered drying to be an important factor in altering the bioactive compounds, as he studied the phenomenon and showed that the selected MAPs had higher levels of bioactive compounds in fresh form than in dried form. He even showed that natural drying of the plants in a room was better than oven drying.

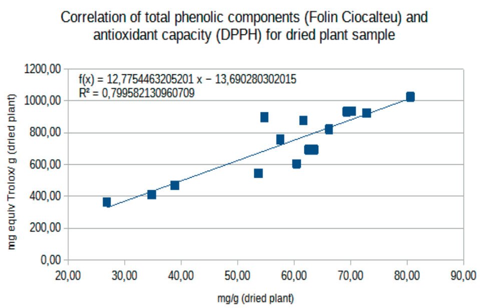

Figure 8. Correlation of values DPPH & TPC (dried plant)

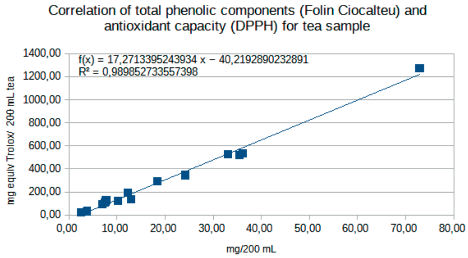

Figure 9. Correlation of values DPPH & TPC (tea)

CONCLUSIONS

The MAPs approach adopted in this study is a contribution to the research field of the evaluation of Mediterranean MAPs, using 5 different flavour propositions belonging to the Mediterranean diet.
The results obtained show that the methods used have the potential to highlight to a large extent the organoleptic and bioactive characteristics of the selected plant species. It is concluded that the combination of these methods can categorise MAPs with a view to their use in urban horticulture. Furthermore, the results showed that some MAPs are an important source of phenolic compounds with high antioxidant capacity and with pleasant acceptance by consumers. Therefore, the consumer can take advantage of these properties by growing the plant in a private space to use it fresh or with controlled drying.
The criteria influencing the organoleptic and quality characteristics of MAPs are discussed in this study. It provides a framework for future research to deepen the multifaceted role that herbs should have in human daily life, urban horticulture, urban planning, and healthy eating. In conclusion, the results of the study offer urban dwellers the opportunity to choose the combination of aromatic/medicinal plants for home cultivation and consumption according to their nutritional and sensory needs.

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THE INFLUENCE OF QUANTUM STRUCTURED WATER ON MICROBIAL DIVERSITY IN RHIZOSPHERE OF TOMATO AND ASSESSMENT OF BIOLOGICAL QUALITY OF PLANTS BY IMAGE-FORMING METHODS

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Abstract

The structured water is obtained by quantum technology, exclusively on the basis of natural elements, under the influence of electromagnetic field with magnitude of the waves in the order of 10^{-40}. The aim of this paper is to present the results of research carried out in greenhouse conditions to assess the influence of irrigation with quantum structured water, as compared with irrigation with tap water, on biodiversity and structure of microbial communities in rhizosphere of tomato (Solanum lycopersicum L.*) FLAVIOLA variety and to evaluate the biological quality of plants. The paper presents the total counts and species of bacteria and fungi estimated by dilution plate method, diversity index of Shannon (H), equitability and similarity index. The influence of structured water on plants hight, fresh biomass accumulated, biological quality and vitality are discussed comparatively with tap water using image-forming methods, namely biocrystallization, circular chromatography and capillary dynamolysis by the evaluation of structures formed consequently to the reaction of plant extracts with certain inorganic salts.*

Key words*: tomato, biodiversity, microbial communities, quantum structured water, image-forming methods.*

INTRODUCTION

Over the recent years, water science has developed enormously in diverse disciplines including physics, chemistry and biology, opening broad perspectives for new tehnological applications, due to the better understanding of its properties.
A comprehensive review (Kontogeorgis et al., 2022) synthesized important information and discussions about water structure, properties and some applications significant for engineering, chemistry, biology and medicine.
Some of novel experiments, theories and inventions refer to the study of "structured water" and the benefits from its applications (Germano, 2015; Dubey et al., 2018).
Recent studies reported the beneficial effect of irrigation with structured water on plants growth and health, yields quality in horticultural plants of tomato, cucumbers, basil, strawberry (Abraham, 2014; Enache et al., 2019a).
A series of picture forming methods are widely utilized in EU countries for acquiring images reflecting the vitality of plants, biological quality assessment of fresh foods, medicaments from medicinal plants, for certification in biodynamic agriculture or differentiation between the efficiency of various agricultural practices.
Thus, circular paper chromatography, capillary dynamolysis or sensitive crystallization as holistic methods, complementary to other methods of quality monitoring, helped successfully in discriminating between various produces from organic and conventional origin (Weibel et al., 2000; Szulk et al., 2010; Fritz et al., 2011, 2020).
A less studied aspect was the application of image-forming method to the assessment of plant quality and vitality under irrigation systems using different types of water.
The aim of the present research was to assess the influence of irrigation with quantum structured water, as compared with irrigation with tap water, on biodiversity and structure of microbial communities in rhizosphere of tomato *(Solanum lycopersicum* L.*)* and to evaluate the biological quality of plants by image forming methods.

MATERIALS AND METHODS

Structured water. The quantum technology, created exclusively on the basis of natural elements was applied to obtain structured water. Tap water was introduced into the apparatus with quantum electromagnetic field with magnitude of the waves in the order of 10^{-40} to obtain structured water. During the water structuring process, the information transported by this electromagnetic field remains captive (stored) in the aqueous physical vacuum from the coherence fields of the water and it is rewritten through the water into the body of the plant.

Experimental variants. The experiment was carried out under controlled conditions of humidity and temperatures (24ºC days and 22ºC night) in Hortinvest greenhouse of the Research Center for Horticultural Products Quality, Faculty of horticulture, UASVM Bucharest, during March-June 2020.

Tomato plants (*Solanum lycopersicum* L.) Flaviola variety with indeterminate vegetative growing and fruit cherry type were watered with quantum structured water (V1). Similar pots were prepared and the tomato plants were watered with tap water (Control). The dose of nitrogen, phosphorus and potassium was the same for both variants.

Plants height (cm) was measured and **total biomass** (grams) **accumulated** was determined by weighing tomato plants from experimental variants.

Microbiological analyses were performed accordingly to soil serial dilution method using specific culture media with agar-agar: Nutrient agar (NA) for aerobic heterotrophic bacteria and potato-dextrose agar (PDA) for fungi. Microbial density was expressed as Total Number of Bacteria-TNB and Total Number of Fungi-TNF reported to gram of dry soil (Dumitru & Manea, 2011).

Taxonomic identification of microbial species in rhizosphere of tomato plants was done using morphologic criteria, according to the determinative manuals (Bergey & Holt, 1994) for heterotrophic bacteria and Domsch & Gams (1970) for fungi. Morphological features were examined and photographed under a MC 5.A optic microscope.

The relative abundance of each species in the structure of microbial community was calculated. Species richness (SR_2 index) in microbial communities from tomato rhizosphere was calculated as the ratio between the total number of species (S) and microbial counts from each experimental variant.

The Shannon index (H') that takes into account both the richness and evenness (ε) of a community was calculated for evaluating the microbial biodiversity in rhizosphere of tomatoes in variant with quantum structured water, as well as in control (tap water) (Mohan & Ardelean, 1993).

The increased value of H' reflects the increased number of species and also the evenly distribution of the species (Morris et al., 2014).

The "equitability" as component of microbial species diversity (distribution of "individuals" on species) was calculated according to Stugren (1982).

Similarity indices (SI) were calculated (Tiwari et al., 1994) for comparative analysis of the microbiome composition.

Picture forming methods used for acquiring new images reflecting the vitality and biological quality of tomato plants were represented by circular paper chromatography, capillary dynamolysis and sensitive crystallization.

Capillary dynamolysis: The differences of biological quality between tomato plants under the influence of experimental conditions of irrigation with quantum structured water and tap water were evaluated by the capillary rising picture method called capillary dynamolysis, according to Kolisko (1953) and refined by Zalecka et al. (2010). A standard quantity of aqueous filtered tomato leaf extract was migrated on a vertical tube of filter paper in a Kaehlin glass dish, followed by intermediate drying, by metal salt (silver nitrate) and iron sulphate migration and development of specific images.

Images of capillary dynamolysis obtained after the development of colors were scanned for analysis of structures using the criteria described by Zalecka et al. (2010), Unluturk et al. (2011) and Böttgenbach (2018).

Sensitive crystallization was another alternative method, setup by Pfeiffer (1984), we used for evaluating the vitality and biological quality of tomato under the influence of tap or quantum structured water by analysis of images of copper chloride crystals formed in contact with leaf extracts to observe the modification of aspect and morphology of the crystallization network.

Circular paper chromatograms were made by migration of tomato leaf extracts through a circular filter paper, previously impregnated by developing substance, to obtain information on biological quality of test plants, according to procedure and recommendations of Pfeiffer (1984).

RESULTS AND DISCUSSIONS

The results of microbiological analyses on total counts of bacteria, fungi and values of indices that characterize biodiversity of each microbial community from rhizosphere of tomato plants from Control and from the variant watered with quantum structured water are presented in Table 1.

Table 1. Total counts and biodiversity indices of bacterial and fungal microflora in rhizosphere of tomato watered with quantum structured water (V1) as compared with tap water (control)

Experimental variant	Bacterial microflora	Fungal microflora
CONTROL (watered with tap water)	TNB=15 x 10^6 viable cells x g^{-1} d.s. S=6 SR_2=0.400 H'=1.714 ε=0.724	TNF=23 x 10^3 cfu x g^{-1} d.s. S=4 SR_2=0.173 H'=1.338 ε=0.695
Variant V1 (watered with quantum structured water)	TNB=12 x 10^6 viable cells x g^{-1} d.s. S=7 SR_2=0.583 H'=1.792 ε=0.701	TNF=15 x 10^3 cfu x g^{-1} d.s. S=7 SR_2=0.466 H'=1.626 ε=0.615
	SI=61.54%	SI=36.36%

Data evidenced moderate densities of bacteria (15 x 10^6 viable cells x g^{-1} d.s. for control and 12 x 10^6 viable cells x g^{-1} d.s. for variant with quantum structured water) and low levels of fungal counts (23 x 10^3 cfu x g^{-1} d.s. for control and 15 x 10^3 cfu x g^{-1} d.s. for variant with quantum structured water).

Microbial diversity was higher in both bacterial and fungal communities from rhizosphere of tomato plants watered with quantum structured water as compared with values of Shannon Diversity Index from control. Thus, a higher number of bacteria species (7) with H'=1.792 and less homogeneous distribution (ε=0.701) were recorded in variant with quantum structured water as compared with control (H'=1.714) but more homogeneous distribution of effectives between the 6 species (ε=0.734).

Fungal community from variant with quantum structured water was more diversified, with 7 species, H'=1.626 and lower homogeneity ε=0.615 as compared with control (4 species, H'=1.338) and more homogeneous distribution (ε=0.695).

Taxonomic composition and the relative abundance values of the bacteria species revealed that the fluorescent pseudomonads were the most abundant in tomato rhizosphere from both control (Figure 1) and the variant with quantum structured water (Figure 2), with A=26.7% and 33.3%, respectively, followed by various species of *Bacillus* and actinomycetes from Series Albus, with lower abundance in population.

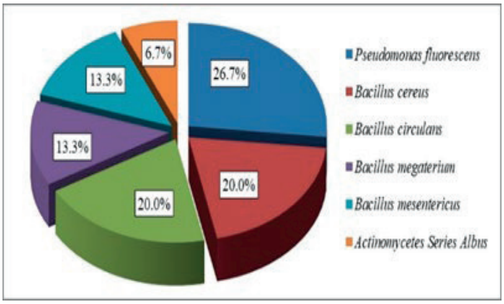

Figure 1. Percent mean relative abundance of bacterial microflora composition in tomato rhizosphere at control (tap water)

The rhizosphere community of fungi isolated from control (Figure 3) was characterized by the dominance of *Fusarium oxysporum* (A=34.8%) and *Penicillium* spp. (A=30.4%), accompanied by less abundant *Aspergillus ochraceus* and *Trichoderma viride* (A=17.4%, each).

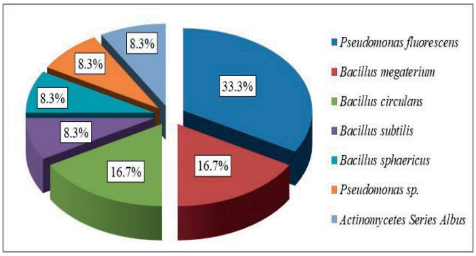

Figure 2. Percent mean relative abundance of bacterial microflora composition in tomato rhizosphere at variant with quantum structured water

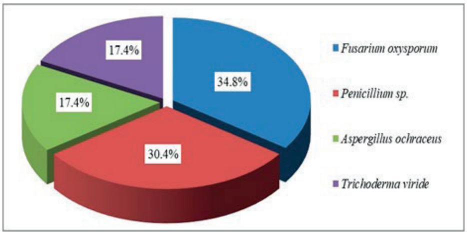

Figure 3. Percent mean relative abundance of fungal microflora composition in tomato rhizosphere at control (tap water)

Watering with quantum structured water increased the biodiversity by stimulating the development of new species of the genus *Penicillium* (e. g. *Penicillium funiculosum,* dominant with A=26.7%, *Penicillium variabile* with A=20%) and proliferation of *Trichoderma viride* with A=20%, higher than in the mycocoenosis from control. Other new species but less abundant in tomato rhizosphere belonged to the genera *Cladosporium, Mortierella, Verticillium* (Figure 4).

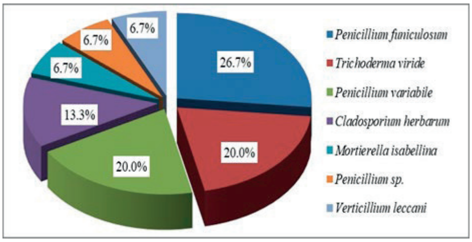

Figure 4. Percent mean relative abundance of fungal microflora composition in tomato rhizosphere at variant with quantum structured water

Similarity Index value SI=61.54% calculated between taxonomic composition of bacterial communities revealed that more than half of species identified were present in both rhizosphere communities. The shared species were *Pseudomonas fluorescens, Bacillus megaterium, Bacillus circulans* and Actinomycetes from Series Albus.

Fungal communities were more different, with a Similarity Index value SI=36.36% reflecting a particular composition and structure and fewer shared species, represented by *Trichoderma viride* and *Penicillium* sp.

In the rhizosphere of plants, soil particles are bound together by phenolics and other exudate compounds released by roots, microorganisms and decomposing organic matter, forming aggregates, thus providing support for plant roots (Makoil & Ndakidemi, 2007).

The majority of microbial species identified in rhizosphere of tomato from our experiment are recognized in literature for the property to perceive signals secreted from the plant root and also to release different signaling molecules for the control of host plant plant growth, root development, as well as for improving biotic and abiotic stress tolerance or eliciting mechanisms of resistance to various pathogens (Weller et al., 2002; Zhang et al, 2017; Yan et al., 2021).

Thus, the results revealing the presence of beneficial species (e. g. fluorescent or non-fluorescent pseudomonads, *Bacillus subtilis*, *Bacillus megaterium*, actinomycetes, *Trichoderma viride,* species from genera *Penicillium, Mortierella*) in tomato rhizosphere are in concordance with research carried out by Jaiswal et al. (2017) who found a link between microflora biodiversity from tomato rhizosphere and suppressiveness effect against various soil-borne pathogens.

Redouan et al. (2018) reported the potential production of volatile and diffusible antifungal metabolites by selected strains belonging to genus *Pseudomonas*, against a wide range of phytopathogenic fungi that infect tomato plants. *Trichoderma* spp. are known from literature for antimicrobial properties against numerous phytopathogens and for a complex enzymatic equipment with role in organic matter recycling (Kucuc & Kivanc, 2003; Gurău et al., 2021; Pani et al., 2021).

Literature data estimated that about 2–5% of bacteria from rhizosphere have plant-growth-promoting traits and are important as potential tools for sustainable agriculture in the future (Alawiy & Babalola, 2019).

Various methods have been developed for improving plants growth, yield, resistance to pathogens or quality. Grafting was reported as a practice meant to increase productivity, or to improve some qualities of tomato fruits such as the soluble dry substance content, the total amount of carbohydrates and vitamin C (Sora et al., 2019).

The influence of quantum structured water was reflected by increased plant hight (138 cm) and total biomass accumulated (991 grams) as compared with the control (123 cm and 916 grams), as presented in Table 2.

Table 2. Plant hight and total biomass accumulated

Experimental variant	Plant height (cm)	Total biomass (grams)
CONTROL (watered with tap water)	123	916
Variant V1 (watered with quantum structured water)	138	991

In 'Flaviola' variety, the sum of the cumulative temperatures and CO_2 had a great influence on the production and quality of tomato fruits (Jerca et al., 2021).
Research results (Jerca et al., 2016) evidenced the influence of watering rate and the type of substrate on the production of tomatoes grown in the greenhouse in unconventional system.
Additional picture (image) forming methods utilized provided new evidences for evaluation of tomato plants quality in control and under the influence of quantum structured water.
Visual evaluation of the paper images formed by **capillary dynamolysis** was carried out evidencing few differences in the aspect of structural elements from base, bowl and flag zone. In both pictures, the three layers of the basal zone are well defined but the colors were more intense at the variant with quantum structured water than in image from the sample watered with tap water.
In control (Figure 5), corona is more irregular, sometimes interrupted by white beards, short flags start from the base and rise not parallel, in apparent disorder, occupying only 1/3 from this zone, ended as thin lines. The apical 2/3 of flag zone are diffuse and the large white zone ended with a thick brown layer, with irregular contour without reduction spots.
In the picture from the variant with quantum structured water (Figure 6), corona is more uniform, without beards. Bowl zone is more diffuse in forms and more colored and continues with a high flag zone containing parallel pipes narrowed towards the drop zone, rarely started from the base, continued in a diffuse grey zone and more often formed on the apical third in the image from the sample watered with structured water. Open ended long pipe in the center of image passes the thin white zone, being in contact with long brown reduction spots coming in single or sometimes in pairs from the thin superior irregular layer.

Figure 5. Capillary dynamolysis of tomato in control watered with tap water

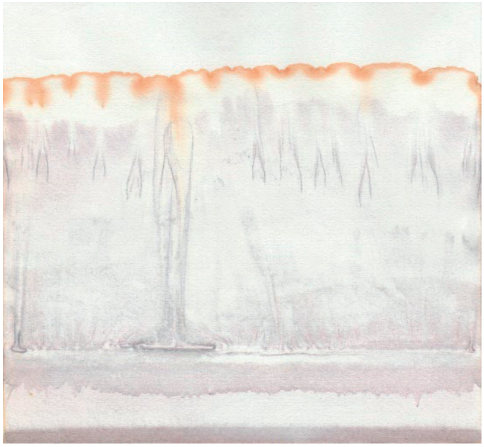

Figure 6. Capillary dynamolysis of tomato in variant watered with quantum structured water

Previous study (Enache et al., 2019b) revealed that irrigation with structured water significantly increased plant height and fresh biomass accumulation as compared to tap water and presented capillary dynamolysis images reflecting the vitality and biological quality of cucumber plants. As in the present experiment, a higher vital force and quality of plants by stronger, more intensely colored and better-defined images were obtained at variants with structured water, more pronounced when added supplementary biological or chemical fertilizers.
Sensitive crystallization generated optimally integrated whole pictures in both control (Figure 7) and in the variant with quantum structured water (Figure 8), showing a center zone located 2-3 cm away from the geometrical center from

which bundles of branches and needles spread towards the margins of the Petri plates.

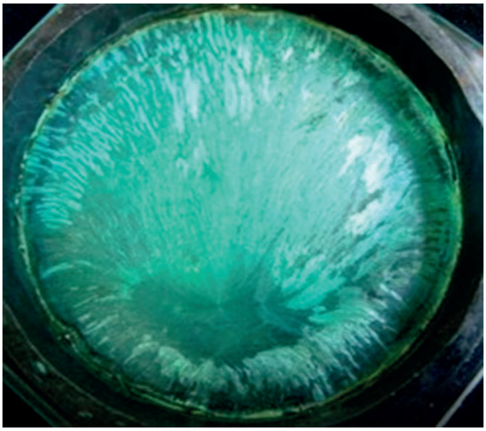

Figure 7. Sensitive crystallization of tomato in control watered with tap water

The two-centered branches and needles from the middle zone are thicker than those from the peripheral zone and more thickened in the variant with quantum structured water than in control. In both crystallization pictures appear numerous curved branches and needles more clearly evidenced in Figure 8.
Similarly, results of Popovic-Vrajes et al. (2016) confirmed that biocrystallisation patterns obtained using crystallisation method, (as a holistic method utilised in order to emphasize the benefits of organic food) evidenced the differences between pasteurized organic and conventional milk.

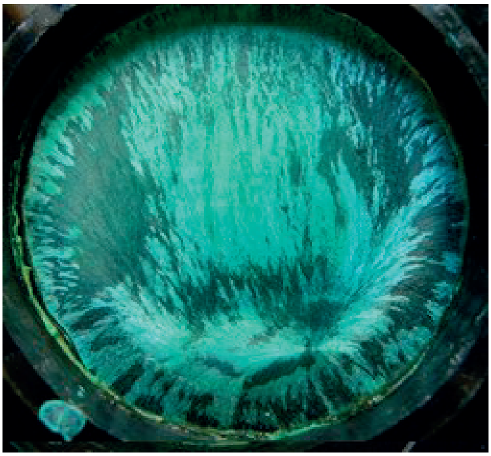

Figure 8. Sensitive crystallization of tomato in variant watered with quantum structured water

Circular chromatograms presented differences of color, general aspect, dimensions of central, intern and especially intermediate zones, as illustrated in Figure 9.

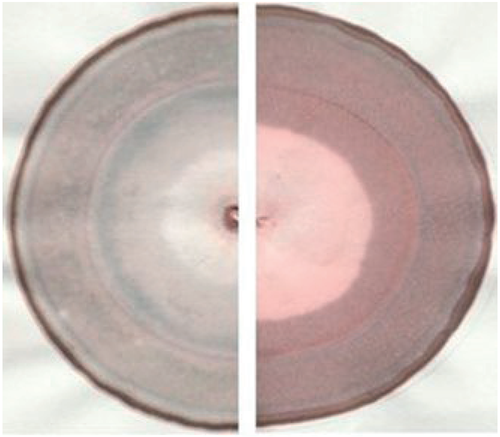

Figure 9. Sections of circular chromatograms of tomato in control (left) and in variant watered with quantum structured water (right)

The chromatogram of tomato plants from the variant with quantum structured water (right) presented lighter colours in reddish brown nuances, better defined forms as compared with more diffuse aspect with blue-grey nuances from control (left).
Our results are in concordance with recent data from literature showing that the three image-forming methods proved to be useful tools for assessing the ageing in terms of degradation (oxidation) of wine from different cultivation systems, completing the sensory analysis (Fritz et al., 2017; 2021).

CONCLUSIONS

Irrigation with structured water significantly increased plant height, fresh biomass accumulation and microbial biodiversity in rhizosphere of tomato plants as compared to tap water.
Differences in the aspect of images obtained by circular paper chromatography, capillary dynamolysis and sensitive crystallization were observed, evidencing a higher vital force and quality of plants watered with quantum structured water.
Information derived from rising pictures analysis proved its usefulness in comparing the

influence of irrigation water on quality of tomato plants.

Further investigations are necessary to link image forming results with usual quality tests for better elucidating significance of differences obtained between control and variant with quantum structured water.

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ENHANCING SOIL SUPPRESSIVENESS WITH CONJUGANTS REALIZED BETWEEN MICROBIAL SIDEROPHORES AND A FULVIC ACID FRACTION

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Abstract

The paper aims to select probiotic microorganisms capable of producing siderophores for iron chelation and to make conjugates with the water-soluble fulvic fraction of Mollic Gleysol (WRB), (0–20 cm). The biosynthetic performances of 5 strains of probiotic bacteria from the collection, belonging to genera Lactobacillus (LAB 41, LAB 62, LAB 57, LAB 83, LAB 69), isolated from different soil types, were evaluated in terms of siderophores production (CAS method), type (Arnow and Csaky tests) and complexation capacity. The probiotic strains produced amounts of siderophores between 97–158 µmol L^{-1} in 48–96 hours, of catecholate and hydroxymate type and of both types in the case of LAB 62, LAB 69 and LAB 83 strains. LAB 83 strain had the highest iron chelating capacity. The inhibition capacity of the conjugates was tested and evaluated on 3 phytopathogenic fungal isolates (P. expansum, A. flavus and A. ochraceus). Probiotic strains are promising for the purpose of producing siderophores but also for antifungal effect when these act as conjugants with a fulvic acid subfraction.

Key words: *siderophores, probiotic bacteria, fulvic acid subfraction, antifungal effect, conjugants.*

INTRODUCTION

In the soil and rhizosphere of plants, the balance between saprophytic and phytopathogenic microorganisms is the determining factor in defining the state of health. In some soils, imbalances favoring the saprophytic component occur so that diseases caused by *Fusarium* spp., *Alternaria* spp, *Rhizoctonia* spp, become unimportant or phytopathogens fail to adapt to those conditions. Such disease-suppressing soils are still insufficiently defined regarding the specific microbial components antagonistic to phytopathogens, as well as the environmental conditions favoring their proliferation. New and relatively effective ways of using agents or their metabolites, in achieving biological control, in order to promote soil and root health, by harmonizing the numerous factors involved in the generation of rhizosphere dynamics, have been constantly developed.

Also, due to the complexity of the interactions of the interdependent factors, simultaneous techniques of stimulation/stabilization of antagonist populations, of root-microbe-microbe interactions as well as intentional alteration of the rhizosphere were used to create unsuitable conditions for phytopathogens.

Suppressive soils offer a natural defense to plants, through the soil microbiota, which constitutes a first line of defense against phytopathogens (Schlatter et al., 2017). In such soils, due to the composition and activity of the microbiota, biosynthesized metabolites, phytopathogens do not establish, cause or not disease, or initially establish in the soil and cause the disease, but then it decreases in intensity, even if the phytopathogens persist in the soil (Weller et al. 2002).

Their combinations generate "general" or "specific" suppressions that ensure in the soil the increase in the level of competitive and collective antagonistic activities of the total soil microbiome, generating competition with phytopathogenic agents. The induced suppressiveness aims to stimulate the pre-existing characteristics in soils, specific to each type of soil and non-transferable.

Probiotic bacterial strains or their siderophores, inoculated in soils with phytopathogens, could intervene by increasing its suppressiveness. Thus, the suppression of various diseases caused by fungal phytopathogens belonging to the genera *Fusarium, Aspergillus, Alternaria, Verticillium* can be obtained by the intake of siderophores from probiotic microbial agents or from their conjugants, through interventions on the efficient complexation of iron (III) in the soil, by causing its unavailability for phytopathogens and inhibiting their growth.

The impact of siderophores on soil iron biogeochemistry may be influenced by the presence of other soil iron-binding compounds such as humic substances (Tipping et al., 1993; 2011; Milne et al., 2011; Niehus et al., 2017; Slagter et al., 2017; Whitby et al., 2020). Studies in the field have highlighted the fact that the distribution of binding sites in organic matter is heterogeneous, relatively similar to those observed in humic substances (Zhu et al., 2021).

Siderophores can act synergistically with fulvic acids (FA) which also function as ligands for the release of Fe from Fe(III) oxides. By combining the two compounds, in different proportions, conjugates can be formed that can adsorb metals more efficiently and release them in larger quantities. The synergistic relationships between siderophores and the water-soluble subfraction of fulvic acids (FA) or the effect of their conjugates, highlight new possibilities regarding the efficiency of Fe (III) acquisition mechanisms in order to ensure its availability for plants and microorganisms, but also to enhance the antimicrobial effects, through which can intervene in the achievement of an effective biocontrol of edaphic phytopathogens.

In this study, the qualities of five probiotic strains of *Lactobacillus* spp. were evaluated regarding the production of siderophores, the formation of conjugates with the water-soluble subfraction of fulvic acids extracted from Mollic Gleysol, and their antifungal character against three fungal species was analyzed (*P. expansum, A. flavus, A.ochraceus*), for a possible application in soils in order to increase their suppressive capacities.

MATERIALS AND METHODS

Screening and growth of probiotic strains. The probiotic bacterial strains come from the laboratory collection and were isolated by the method of serial dilutions using MRS (De Man Rogosa Sharpe) agar medium, from two types of soil according to WRB (Haplic Chernozem, Rendzic Leptosol). Plates were incubated at 27°C for 72 hours, identified morphologically and characterized by biochemical tests. The strains were preserved and maintained on MRS broth at 4°C. Morphologically, the strains LAB 41, LAB 57 presented rough, yellow, irregular surfaces and LAB 62, LAB 69 and LAB 83 presented smooth, white, circular surfaces, Gram-positive, by the Gram staining method.

Qualitative detection of siderophores production was performed using the CAS (Chrome Azurol S) assay according to Schwyn & Neilands (1987). The culture supernatant obtained was mixed in equal volumes with CAS reagent and the color change observed. The reference was prepared on the basis of the uninoculated medium, as a control. Through the qualitative determinations, the production of siderophores was estimated for a number of 12 isolates and of these, five probiotic bacterial strains, LAB 41, LAB 62, LAB 57, LAB 83, LAB 69, were selected for the following tests, due to their increased synthesis capacities.

Quantitative detection of siderophores. The chromium azurol S (CAS) method was used to estimate bacterial siderophore production due to its detection capability (Alexander & Zuberer, 1991). The culture media were centrifuged, the supernatant filtered through a 0.45 μm filter, stored at low temperature. Afterwards, 1.0 mL of filtrate was mixed with deionized water and 1 mL of test solution. For the reference solution, 1.0 mL of CAS solution was mixed with 1.0 mL of deionized water and for the zero absorption solution, 1.0 mL of CAS solution with 1.0 mL of deferoxamine, kept at room temperature, in the dark, 24 hours and read the absorbance at 630 nm. Siderophore production, according to the calibration curve, was expressed as μmol L^{-1} deferral equivalent. For each probiotic isolate, samples were read in triplicate.

Determination of the type of siderophores. synthesized by the selected strains was carried out using the Arnow test, for the catecholate siderophore type and the Csaky test, for the hydroximate siderophore type.

Conditions for the synthesis of siderophores. Factors were similar for siderophore production in all strains (incubation time, pH, rpm, inoculum volume). The incubation period was 96 hours using the CAS assay and pH for growth of strains in medium was 7-8 using identical molarities for HCl and NaOH. Agitation at 180 rpm and 2% inoculum volume was used for siderophore production.

Siderophore extraction. After completion of incubation period, cell mass from each five culture strains were separated by centrifugation at 10.000 rpm, 4ºC for 15 min and supernatant was concentrated. Siderophores were extracted in two phases with phenol: chloroform, diluted with diethyl-ether and transferred to aqueous phase.

Complexation capacity assays. Siderophore complexation capacity was assessed following a method adapted from Villen et al. (2007). To a constant volume of culture filtrate, FeCl3 was added, the pH adjusted to 9.0, for 30 min. The obtained solution was centrifuged, filtered through a 0.45 μm pore membrane and the amount of Fe in the solution was determined and presented graphically.

The Fulvic water-soluble subfraction Fulvic fraction from Mollic Gleysol was extracted and fractionated by adsorption on activated charcoal and serial elutions with acetone, NaOH of the absorbed fulvic substances and with distilled water for separating water-soluble subfraction (Votolin et al., 2022).

Specific ascending chromatograms Quantitative/qualitative changes of fulvic subfraction and the degree of complexity reached was obtained by using specific ascending chromatogram adapted method (Mukadam et al., 2021).

The formation of image pattern of uniformity, shape, size and color could directly highlight the variation of composition of labile fulvic compounds, in a short period of time.

Conjugates siderophores-fulvic subfraction Siderophore-fulvic subfraction conjugates were designed to intensively depriving the phytopathogens from iron, as antimicrobial strategy, based on conjugants features, which were enabled for a more active iron uptake than individual components and for using as supplementary weapon in antifungal treatment.

Fluorescence of conjugates The siderophores-fulvic subfraction conjugants were treated with fluorochromes and their distribution was revealed (Wang et al., 2021). Thus, photographic images were obtained under UV illumination of 350 nm, which revealed the quantitative differences between conjugants, related to the density of the newly biochemical compounds, in close agreement with fluorescence-based affinity.

Antifungal activity. Agar well diffusion assay was used to detect the antifungal activity of the conjugates against phytopathogens (*P. expansum, A. ochraceus, A. flavus*) grown on PDA (potato dextrose agar) medium. The agar plate surface was inoculated by spreading a volume (1 ml) broth culture of the phytopathogen inoculum (10^3 ufc x ml^{-1}) over the entire agar surface, incubated at 27°C for 24 hours. 50 μl of conjugants were placed in agar wells, incubated at 27°C, five days and measured the diameter of inhibition zone around the wells.

RESULTS AND DISCUSSIONS

The colonies of bacteria developing a yellow halo on CAS medium (Figure 1) were selected and isolated from two types of soils (Haplic Chernozem, Rendzic Leptosol) for producing siderophore.

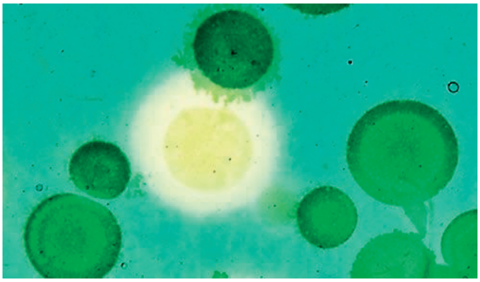

Figure 1. Isolation and selection of siderophores-producing bacteria (colony with yellow halo)

Initially, twelve probiotic bacterial isolates from the two soils were used for screening to siderophore production capacity, both qualitatively and quantitatively.

Five efficient bacterial strains were selected and accepted to be analyzed in terms of siderophore type, production and complexation capacity, in order to be used in increasing the suppressiveness of soils.

All efficient probiotic strains were grown at 27°C with shaking in MRS broth. Pre-cultures

were prepared in media with normal iron content.
The cell mass of the probiotic bacteria was separated, washed and re-inoculated in the same type of medium, but without optimal iron content. All operations were carried out during the exponential growth phase, to limit the transfer of iron from the iron medium, as it could intervene by inhibiting the production of siderophores. At the end of growth, iron was below the detection limit in the culture medium. All probiotic strains grew in the medium and had different doubling times. In the stationary phase of growth, LAB 41 and LAB 57 strains grew more slowly in the culture medium. Under the culture conditions, they showed a doubling time of 18 hours and were in stationary phase after 48 hours. The probiotic strains LAB 62, LAB 67 and LAB 83 grew faster in the culture medium and were in stationary phase after 24-36 hours (Figure 2).

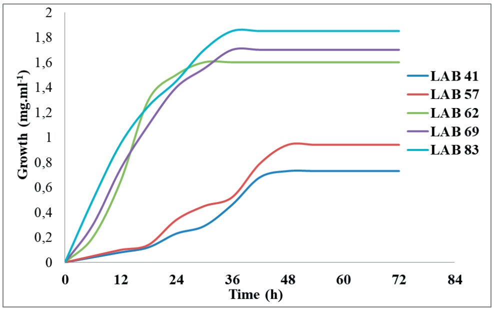

Figure 2. The growth of probiotic bacteria

In the first stage, qualitative screening of probiotic strains has been performed using CAS agar plates. So, all five strains that formed a yellow to orange zone around the well as seen in Figure 3 indicated a positive siderophore production. All strains tested were positive for siderophore production, the CAS agar method providing only an approximation and not an accurate quantification of siderophore production. In the agar well diffusion test, when supernatant of each probiotic broth culture were applied to the wells, the appearance of a halo yellow to orange around the wells indicates the production of siderophores.
The halo diameter varied from strain to strain and was considered direct proportional with the content of synthetized siderophores. The best performance were obtained by five probiotic strains which used forwards in making conjugates. The strain LAB 83 determined the highest diameter of the halo to 28.6±0.26 mm and the strain LAB 57 determined the lowist diameter of the halo to 12.4±0.11 mm. The probiotic strains LAB 41, LAB 62 and LAB 69 determined intermediate values of the halos diameters (Figure 3).

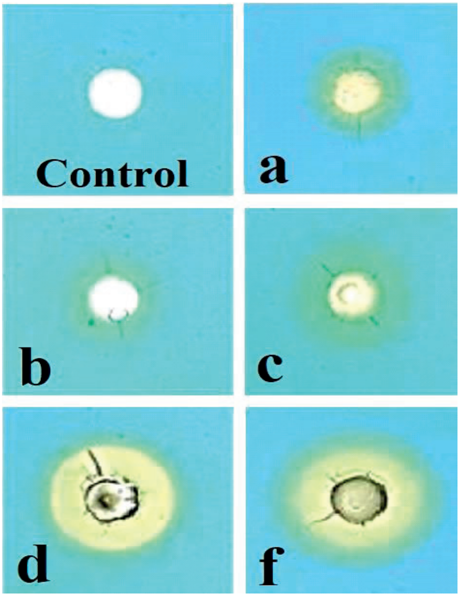

Figure 3. The halo diameter in direct relation with the content of synthesized siderophores by a) LAB 41, b) LAB 57, c) LAB 69, d) LAB 62, f) LAB 83

The determinations revealed that the LAB 83 strain was the most effective in terms of qualitative evaluation of siderophore production, compared to the other probiotic strains. Based on the diameters of the halo, the strains were grouped into three levels of siderophore production (Table 1).

Table 1. Siderophore production by probiotic bacterial strains and their estimation using qualitative analysis

Probiotic bacterial strains	Qualitative siderophores analysis
Control	-
LAB 41	++
LAB 57	+
LAB 62	++
LAB 69	++
LAB 83	+++

The production of siderophores is a specific feature of many groups of edaphic microorganisms, soil bacteria and fungi, but also phytopathogenic agents. The production of siderophores by the analyzed edaphic bacteria highlights an important feature of them that may suggest the possibility of involvement both in growth promotion and in the biocontrol of phytopathogens (Johnstone et al., 2015; Koh et al., 2015; Duar et al., 2017; Senthilkumar et al., 2021). Our results highlight that siderophore production occurs on a large scale by soil microbiota and many of the probiotic bacteria produce and utilize siderophores. Siderophore production of probiotic bacteria was monitored and compared, at different time intervals, using the CAS method. In the culture conditions tested, the concentration of siderophores determined in the culture filtrates of *Lactobacillus* spp. reached the maximum level at 96 hours, when they were in stationary phase. After this time interval, the level of siderophores remained relatively stable. According to the same model, siderophores synthesized in culture filtrates LAB 62, LAB 69, LAB 83 reached the maximum level at 48 hours. The concentration of siderophores in the filtrates was relatively stable after 32 to 48 h of growth, followed only by a variation in the values after 48 h. In the case of the LAB 83 strain, a further increase in siderophore production was observed even after 56 hours (Figure 4).

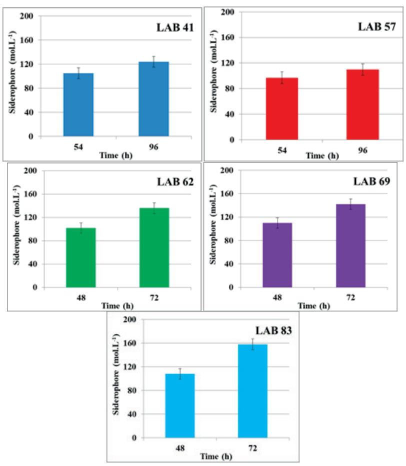

Figure 4. Siderophore production of probiotic bacteria using the CAS method

In the present investigation, the five probiotic bacterial isolates of *Lactobacillus* were tested for 2 different types of siderophore. All isolates (LAB 41, LAB 57, LAB 62, LAB 69, LAB 83) were positive in Arnow's test that detects the catechol type of siderophores. Three isolates (LAB 62, LAB 69, LAB 83) tested positive in Csáky's test which detects the hydroxamate type of siderophores (Table 2). No other determinations were made for other types of siderophores (carboxylate).

Table 2. Analysis of the siderophore-type produced by the probiotic bacteria

Probiotic bacteria	**Arnow[a]**	**Csaky[b]**
LAB 41	+	-
LAB 57	+	-
LAB 62	+	+
LAB 67	++	+
LAB 83	++	+

Catechol[a] and hydroxamate[b] - type siderophores were identified using Arnow[a] and Csaky[b]s tests, with ++ strong positive; + positive; – negative result

Hydroxamate siderophore units present a broad spectrum of antibiotic activity, through bactericidal units that can inhibit tRNA synthetase, similar to fungal ferrochromes, due to the presence of ferrochrome receptors in both Gram-positive and Gram-negative bacteria. These naturally occurring “Trojan horses” formed the basis for the development of synthetic antimicrobial conjugates (Ji et al., 2012; de Carvalho et al., 2014). Siderophores exhibiting hydroxamic acid groups for metal binding and containing additional cyclic structures have the potential advantage of conjugate stability over a wide pH range, enhancement of specific activities and high stability at low conjugate concentrations. Thus, trihydroxamate siderophores (deferoxamine B) act synergistically with fulvic acid, as a low molecular weight organic ligand, to release Fe from Fe (III) oxides. Desorption and release from conjugates can be influenced by the type of siderophore, the composition of the fulvic acids subfraction, as well as their combined effect.
Selected probiotic bacterial filtrates could be used to make siderophore conjugates for soil amendment. Thus, in order to assess the iron chelation potential of the culture filtrates of the selected bacterial probiotics and the influences

due to the culture conditions, they were subjected to complexation capacity analyses (Payne et al., 1993; Perry et al., 2015; Roosenberg et al., 2000; Souza et al., 2017). The results of these analyzes on the complexing capacities are presented in Figure 5. For strains LAB 62, LAB 69 and LAB 83, the initial slopes of the experimental data are close to 1 up to a [Fe] added/Siderophore ratio of about 1.1-1.2, indicating effective and good complexation to the amount of added iron. In the probiotic bacterial strains LAB 41 and LAB 57, delays are observed, the change from the slope = 1 appears in the [Fe] added/Siderophore ratio values of 2.4-2.7, showing an efficiency of approximately 33-45%. Deviations may be due to inefficient complexation in the pH range used, complexation of siderophores/chelating agents with weaker characteristics, to which the added iron cannot be efficiently complexed or may precipitate. The average of iron complexed compared to that of added iron, depending on the total concentration of siderophores, was determined by the CAS method. For each filtrate, based on the CAS values, the amount of effectively complexed Fe was estimated. For example, in literature was describes that *R. radiobacter* produces agrobactin, which complexes iron in a 1:1 ratio, in a hexadentate conformation; thus, a slope close to 1 would be expected as a result of high complex stability and consequent efficiency of complexation.

The observed results may be due to the different efficiency of iron chelation under the established conditions, as well as the presence of siderophores with different stoichiometry and stability. Thus, in the case of the filtrate of LAB 57, taking into account a CAS reading, we detect 97 µmol L^{-1} of dissolved iron upon the addition of about 262 µmol L^{-1} of Fe. A filtrate of LAB 83 was able to completely dissolve 158 µmol L^{-1} of Fe by adding 191 µmol L^{-1} of Fe. The observed results may be due to different iron chelation efficiency under the established conditions, stoichiometry and specific stability.

Fulvic acids and its water-soluble subfractions constitute novel antimicrobial molecules reported to have antifungal properties and the ability to bind and eliminate toxins in soil, such as heavy metals, making it a powerful detox ally compound.

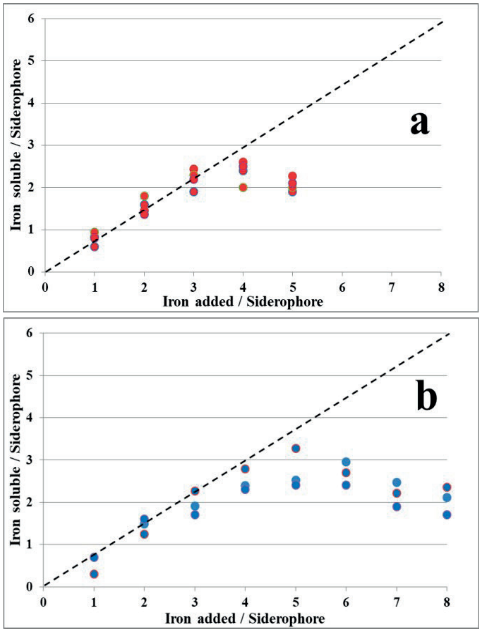

Figure 5. Estimation of the amount of effectively complexed Fe in relation to the values of Fe added / Siderophores, based on the CAS values a) LAB 83, b) LAB 57

The change in the structure of organic compounds in soils, especially of the fulvic acid (FA), determines intense interactions with the plant growth process and the activity of microorganisms.

There are relatively few studies on the antifungal activity of fulvic compounds, especially FA subfractions against plant phytopathogenic fungi.

Organic compounds (humic or fulvic precursors) extracted from soils, as well as synthetic ones show antimicrobial activity, compared to those extracted from peat or coal which insignificantly inhibited the bacterial microbiota (e.g. *Streptomyces pyogenes, Pseudomonas aeruginosa*).

But, by simple changes regarding the initial chemical structure of the organic acids (by acylation), it is possible to increase their biological activity.

Generally, fulvic acids (FA) made favorable associations to soil sustainable characteristics, determining a better water penetration in compacted soils, root growth and development, increase organic matter content, root size, water retention, retain or leach plant nutrients.

(Canellas et al., 2002; Li et al., 2019; Dariellys et al., 2014; Just al., 2021).

The ascending chromatograms of the water-soluble fulvic sub-fraction of Mollic Gleysol revealed the qualitative structure and organizing of the organic compounds with low molecular weigh.

The organic material accumulations in the fulvic sub-fractions, exprime the potential of the microbiome to bio-synthesize secondary exometabolites, biosynthesis specific to each type of soil.

The chromatogram made possible to distinguish different characteristics, based on the specificity of the type of microbiome, as well as indications on the complexity and on an effect probable to making the conjugates by specific features to the ligand organic compounds, with characteristics induced by the microorganisms.

The biosynthesis of low molecular weight organic compounds, especially water-soluble, with specificity to each type of soil could be it considered effective becouse react with the minerals very quickly for the organic matter stabilization.

In subfraction of Mollic Gleysol (Figure 6), the elution chromatogram showed, after absorption of the extract of the fulvic subfractions on activated carbon, the accumulations of organic compounds soluble in acidified water, with relatively low molecular weights, such as carbohydrates and amino acids.

The structuring of the compounds of the fulvic sub-fraction appear on the background of a richer and more diversified organic matter.

Also, in sub-fraction, some accumulations of water-soluble organic compounds, such as polysaccharide compounds, mono-carbohydrates, amino sugars, were well highlighted in the case of soil.

After dialysis, the chromatogram revealed a colour content, formed mainly by colored compounds such as amino sugars, pentose, organic compounds with phosphorus, well highlighted the accumulations of organic compounds soluble in alkaline water, with relatively higher molecular weights.

The chromatogram of fulvic sub-fraction showed the structuring of it compounds, the formation of the short-acting and highly mobile compounds. The coloring suggest an intense biosynthesis of such mobile organic compounds.

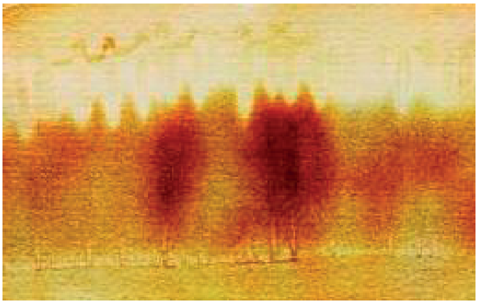

Figure 6. The image of ascending specific chromatogram of the alkaline water-soluble fulvic subfraction from Mollic Gleysol

The formation of complexes with inorganic constituents and the evolution to compounds with insoluble character indicate a intensification of association reactions. Thick migration fronts and dark external areas appear, if in the extract, the concentration of fulvic compounds is high. The fulvic sub-fraction show an increase of the mineral diversity in soil, relatively integrated in the organic material, structural complexity increases with the high level from microbial activity.The increase in intensity of microbial activities maintains the accumulation and integration of biosynthesized organic material.

Mainly, the stabilization of microbial metabolites on mineral surfaces influence the formation of stable organic matter in the soil (Li et al., 2019; Giannetta et al., 2019; Just et al., 2021; Kleber et al., 2021; Song et al., 2022). Organic compounds linked come mostly from microbial metabolism, appear layered on the clay particles and stabilized, due to the content in proteins and polysaccharides of the microbial residues.

In Figure 7 were presented the conjugates between water-soluble fulvic subfraction and siderophores from each strain of probiotic bacteria (LAB 41, LAB 57, LAB 62, LAB 69, LAB 83). The fluorescent highlight of the conjugates was proportional with chemical characteristics and specific production of siderophores.

The advantages offered by siderophore-fulvic subfraction conjugates include increased antifungal potency, improved selectivity for phytopathogens, turning them into susceptible microorganisms. Perhaps such high molecular weight conjugates should be considered because large molecules can be tolerate, can be effective, relatively similar to the mode of action of natural antibiotics (Blaskovich et. al., 2015). In addition, it would be important to make more conjugates with structurally diverse natural siderophores as more than 500 siderophores have been reported (Hider & Kong, 2010). Possibly, such conjugates cannot be considered definitive solutions regarding the presence/persistence of phytopathogens, but important molecular structures in the fight, through their antimicrobial potential (Ouchetto et al., 2005; Noel et al., 2011).

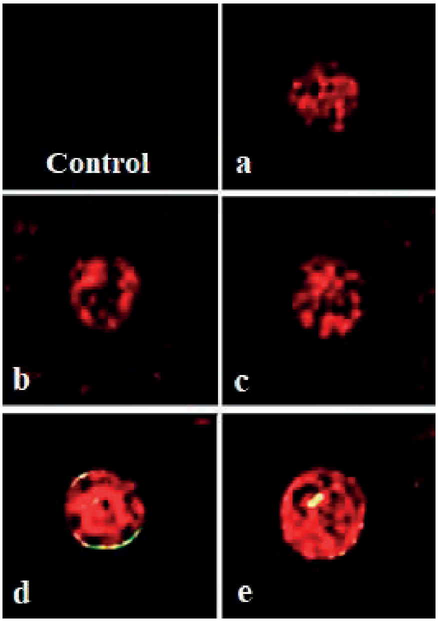

Figure 7. Fluorescent highlighting of conjugates between water-soluble fulvic subfraction and siderophores from a)LAB 41, b) LAB 57, c)LAB 62, d)LAB 69, e)LAB 83

After five days of incubation, the antifungal activity of the conjugates against phytopathogens (*P. expansum, A. ochraceus, A. flavus*) was evaluated by measuring the diameter of the inhibition zone around the wells. The agar plates were photographed and later analyzed. The inhibition zone was measured (mm) on the plates with the dispersed phytopathogens to estimate the antifungal activity. The inhibition of the phytopathogens analyzed was the lowest in the case of the bacterial strain LAB 57, the diameter of the inhibition zone being 11.6 mm for *P. expansum*, 12.4 mm for *A. flavus* and 12.7 mm for *A. ochraceus*. The strain LAB 83 determined the strongest inhibition of phytopathogens, the diameter of the inhibition zone being 24.5 mm for *P. expansum*, 22.3 mm for *A. flavus* and 20.2 mm for *A. ochraceus* The values of the inhibition zones determined for the other probiotic strains were intermediate (Figures 8).

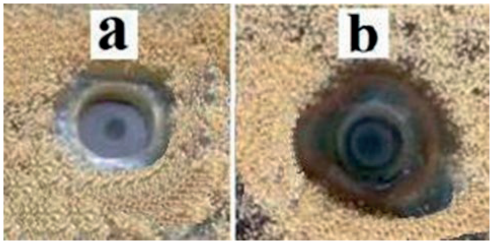

Figure 8. Inhibition zone of *A. ochraceus* induced by the conjugates between fulvic subfraction and siderophores from a) LAB 57, b) LAB 83

Probiotic bacteria possess plasma membrane-localized siderophores, consisting of ligands that form complexes that cover ferric ion coordination sites and achieve increased iron binding constants. For phytopathogens in different plant hosts, iron is no longer available, but tightly sequestered. Blocking access to iron by invading phytopathogens triggers the host's defense response by creating nutritional immunity (Nazarov et al., 2020). As a result, phytopathogens had to develop sophisticated strategies to ensure iron supply and the control of absorption can be considered a major homeostatic mechanism of them.
Probiotics directly intervene in the biocontrol of phytopathogens by modulating nutrient absorption (phosphorus and potassium), hormone production, nitrogen fixation and siderophores. Through the diversity of compounds with an antimicrobial role, probiotics also intervene indirectly in the biological control of phytopathogens by causing reactions to induce systemic resistance, mechanisms involved in the attack, as well as by decreasing the availability of iron for the phytopathogen (Sathe et al., 2007; Fhoula et al., 2013; Guo et al., 2013; Sangmanee and

Hongpattarakere, 2014; Cortes-Zavaleta et al., 2014; Mislin et al., 2014;Lamont et al., 2017, Kharazian et al., 2017; Juodeikiene et al., 2018; Arena et al., 2019; Sadiq et al., 2019; Muhialdin et al., 2020; Chen at al., 2021; Patel et al., 2021; Dopazo et al., 2022; Jaini et al., 2022).

The siderophores produced by the selected probiotic bacteria help plants to obtain iron requirements from the environment. Its absorption also acts antagonistically to phytopathogens in the soil by limiting its availability.

Studies have highlighted their inhibitory potential against phytopathogens, based on the ability to produce siderophores. Thus, bacterial probiotics show an antifungal effect against *Z. tritici* and the species *Lactiplantibacillus plantarum* exerts a strong antagonism against the necrotrophic fungus *Botrytis cinerea* (Lynch et al., 2016; De Simone et al., 2021).

Species of the *Lactobacillus* genus also show antifungal activity against *P. digitatum* and other phytopathogenic species (Matei et al., 2019).

Also, an antifungal effect of probiotic strain S2 filtrate against *Aspergillus flavus* was reported by affecting fungal growth, spore numbers (with two orders of magnitude), morphology, the level of aflatoxins produced (Al-Haik et al., 2017).

Due to the function of virulence determinants, probiotic bacteria can develop a diverse range of siderophore sequestering compounds, and in response, phytopathogens create mechanisms to avoid siderophore recognition by sequestering compounds (Sia et al., 2013; Koh et al., 2015).

The involvement of probiotic bacteria, as potentially useful agents against phytopathogens, results from the biosecurity elements and from the way of use in the biostimulation of some processes (Visser et al., 1986; Higa & Kinjo, 1989; Gajbhiye & Kapadnis, 2016; Daranas et al., 2019; Haryadi et al., 2019; Lim et al., 2019; Alexander et al., 2021; Duha & Abdullah, 2021; Malik et al., 2021;Abhyankar et al., 2022; Jaini et al., 2022). Thus, their GRAS status and history in food research make them ideal for use in biocontrol.

CONCLUSIONS

It was highlighted that the strains LAB 62, LAB 69 and LAB 83 produced siderophores in large quantities, under the established conditions, after an incubation period of 48 - 96 hours.

All five isolates produced catecholate-type siderophores, and of these, three strains also produced the hydroxymate type, under iron stress conditions.

The selected probiotic strains of *Lactobacillus* spp. produced siderophores in vary quantities.

Our isolates were shown to be potent siderophore-producing probiotic strains capable of forming conjugates with the water-soluble fulvic subfraction of Mollic Gleysol.

Siderophore-fulvic subfraction conjugates, through the effect of inhibiting phytopathogenic agents, make it possible to apply and increase the suppressive capacity of soils.

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EVALUATION OF PHENOTYPIC EXPRESSIVITY OF *SIDERITIS SCARDICA* VAR. OSSA, A NEW GENOTYPE ACCLIMATED AND BRED IN ROMANIA

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Abstract

BRGV Buzau owns a valuable collection of genotypes belonging to the Sideritis species, including the varieties: scardica, syriaca, hyssopifolia. S. scardica var. Ossa, coming from the mountain bearing the same name, has medicinal, gastronomic, pharmaceutical and ornamental value. Since 2002 it has been studied by researchers from Buzau and since 2019 it has been intensively introduced into cultivation at BRGV Buzau. The research has shown that var. Ossa has an increased ecological plasticity to the soil and climate conditions in Romania. Special attention was paid to the biological isolation of the genotypes, knowing that it is an entomophilous plant preferred by insects, especially bees that can easily pollute the studied genetic resource. Following the evaluation of the BRGV collection, the G2 genotype was retained, which is the subject of this article. G2 shows distinct phenotypic expressivity, genetic stability and, very importantly, a strong, specific aroma. During the research, biometric determinations and phonological observations were carried out, with average values of 708g leaf mass and 278 g flower stems, 32 cm bush height, 71 cm diameter.

Key words: *entomophily, ecological plasticity, habitat, germplasm collection, genetic stability.*

INTRODUCTION

Sideritis scardica Griseb is an outcrossing diploid (2n = 32) (Esra et al., 2009) and perennial subalpine/alpine herbaceous plant, endemic to the central parts of the Balkan Peninsula (Grdiša et al., 2019). The species is distributed in southwest parts of Albania, North Macedonia, Bulgaria and Greece (Petrova et al., 2009). It is a plant of the alpine zone, occurring in dry stony meadows, mainly at altitudes of 1600-2300 m a.s.l., and only occasionally down to 500 m, a.s.l. on limestone (Strid et al., 1991; Papanikoulau et al., 1982). *Sideritis scardica* Griseb var. Ossa originates from the mountain of the same name, Ossa, in northern Greece (Papanikoulau et al., 1982), being a genotype with very valuable distinct phenotypic characteristics. Manel et al., 2012, suggested that genetic variation that appears to be caused by natural selection might be the results of isolation by distance, which limits gene flow among populations or the result of secondary contact of populations that survived isolated in glacial refugia. The local population of *Sideritis scardica* var. Ossa is located on Mount Ossa, at 1200 meters altitude. Recently, "The Ossa - Herbal gardens" farm was established here, which organically cultivates two species of aromatic plants: *Sideritis scardica* and *Origanum vulgare* and markets the products under the name Ossa tea (Figure 1) and Ossa oregano, respectively.
In Romania, it was taken into study in 2002 by researcher Costel Vînătoru, who in 2019 obtained the first variety of *Sideritis scardica*, patented and registered in the Official Catalogue of Cultivated Plants in Romania under the name of Domnesc (Vînătoru et al., 2019). Research continued at Plant Genetic Resources Bank (PGRB) Buzau, with the aim of enriching the germplasm base of the *Sideritis* species and developing the cultivation technology specific to this perennial crop. This article presents the studies carried out on genotype G2, *Sideritis scardica* Griseb var.

Ossa, and the evaluation of its phenotypic characters with the aim of obtaining a new genotype with distinct phenotypic expressiveness acclimatized to Romanian soil and climatic conditions.

Figure 1. Culture of *Sideritis scardica* var. Ossa from Mt Ossa and the commercial product of "The Ossa - Herbal garden" https://ossaherbs.gr/mountaintea.html

MATERIALS AND METHODS

The research works were carried out at PGRB Buzau (45°09´N, 25°5´E, 95 m) located in SE Romania, characterized by continental climate, steppe characteristic vegetation and leached chernozem soil.

The period analysed in this study was 2019-2022.

The genetic resources of *Sideritis scardica* var. Ossa come from the PGRB seed collection and purchase from the area of origin. Because of the very low germinations ratio of under 27, the seeds were purity tested in the Seed Conditioning and Analysis PGRB Laboratory and were sown in the greenhouse using 80% peat and 20% dolomite, which alkalizes the substrate creating premises for better germination. By comparison, the seedlings of *Sideritis scardica* var. Domnesc had germination between 80 and 95%, with the same type of substrate being used (Figure 2).

a) b) c)

Figure 2. Seedlings of *Sideritis scardica*: a) var. Domnesc, germination approx. 80%; b) var. Ossa germination< 27%; c) seedling of var. Ossa - detail

The sowing was done in the first decade of March in protected spaces. The crop was established by direct sowing, after land modeling using the L445 tractor in aggregate with MMS 2.8 with 140 cm gauge, 94 cm canopy length, 46cm gutter and 70 cm between rows. About 60 days after sowing, the culture was established through seedlings around 10.05.2019, at the stage of 6-8 leaves and 7-8 cm in height (Figure 1).

The breeding methods used were repeated individual selection followed by negative mass selection. Phenological observations and biometric measurements were carried out according to the UPOV and IPGRI data sheets. Weighing operations were performed at PGRB laboratory on green plants and the device used was the two-decimal precision balance Kern 572-33.

RESULTS AND DISCUSSIONS

Sideritis scardica Griseb var. Ossa prefers rocky soils with sandy-clay composition, pH between 6.9-8 and low nutrient content. The crop doesn’t require special handling because it originally grows in mountainous, sloping, stony soils, in the high mountains (Kloukina et al., 2020). On the other hand, observations in Rhodopean locations (Bulgaria) have shown that *Sideritis scardica* inhabits dry, sunny, grassy, and stony terrains, solely on a calcareous rock base. That is why a number of its morphological characteristics relate to its adaptation to permanently scanty humidity typical of the karst massives, namely a powerful rootsystem and profuse hairiness (Yordanova & Apostolova, 2000).

During the study, we did not detect any diseases and/or pests that would cause significant damage to the crop. *Sideritis scardica* Griseb is a perennial herbaceous plant from Ossa Mountain. Through the breeding methods used, repeated individual selection followed by negative mass selection, the G2 variety presented in this article was obtained.

It should be noted that in the first two years of cultivation the two genotypes of *Sideritis scardica,* var. Ossa and var. Domnesc, are very similar in morphology and the difference lies in the development of the bush in the horizontal plane.

a) b)

Figure 3. The two *Sideritis scardica* varieties in the second year of cultivation: a) var. Ossa; b) var. Domnesc

More precisely, the diameter of the shrub var. Ossa is between 12-15 cm, and that of var. Domnesc is between 25-35 cm (Figure 3).

The species shows a valuable characteristic, namely branching, which materializes in the formation of several stems and flower shanks. The bush has a lignified stem from the base to the level of the secondary and rarely tertiary branches, which gives it resistance to low temperatures, strong winds, and large temperature fluctuations in the day/night interval. The bush is erect and the port is small compared to *Sideritis scardica* var. Domnesc. In the third year of culture, the plant has a maximum height of 29-34 cm and a diameter of 68-74 cm. *S. scardica* in Slavyanka Mt. origin area has a plant diameter between 25-90 cm, with between 2-27 flowering stems each. The number of flowers in one stem varies from 12 - 84. The number of seeds in one flower is 4 (Aneva et al., 2013). The G2 genotype of *Sideritis scardica* from our study has 187-195 secondary and tertiary branches, with between 3-7 inflorescences each, in the third year crop (Figure 4).

Figure 4. *Sideritis scardica* var. Ossa, third year of culture

The basal leaves are moderate thick, oblong-lanceolate, usually crenate, mucronate, attenuate to a petiole; the length of basal leaves varies between 5.7-6.3 cm, the width between 0.9-1.2 cm, and the thickness between 1.1-1.16 mm; middle and upper stem leaves are subsessile, linear-lanceolate; their length varies between 2.8-3.7 cm, the width is between 0.3-0.7 cm and the thickness between 0.6-0.8 mm (Figure 5).

Figure 5. *Sideritis scardica* var. Ossa, detail of linear-lanceolate narrow leaf

Leaves are uniformly silver-green due to the presence of glandular hairs distributed equally on the foliage and stems. The small dimensions of the leaf apparatus in var. Ossa is reflected in the number of leaves per stem, on the one hand, which varies between 8-22, and the distance between leaves, on the other hand, which is between 4.5-5.5 cm. In *Sideritis scardica* var. Domnesc, the leaf apparatus is more developed, the distance between leaves on the stem is bigger, 6-8 cm, the number of leaves per stem is between 6-10, but leaves are double in size: leaf length 5.1-10.9 cm, width 0.7-1.3 cm and thickness 0.8-1.6 mm.

Var. Ossa inflorescences are herringbone-shaped with pointed, stellate bracts, medium bristles and yellow flowers. Vertices are dark green, small in number and spaced, which is a characteristic of this variety (Figure 6). The first two vertices are spaced at 4 cm and 3 cm respectively, narrowing the distance between vertices towards the tip of the inflorescence, where they are more compact. On average, the number of vertices in var. Ossa is between 3-11 and in var. Domnesc is between 17-21. The picture below shows the difference between the inflorescences of the two *Sideritis scardica* genotypes, var. Ossa with lax inflorescence with sparsely arranged vertices and dense glandular hairs, which gives the silver-green color, and var. Domnesc with twice the number of vertices, compactly arranged along the entire length of the inflorescence, and less numerous glandular hairs on the surface of the bracts, which gives a deep green color.


a) b)
Figure 6. *Sideritis scardica*: a) var. Ossa, lax inflorescence; b) var. Domnesc, compact inflorescence

Inflorescence is extremely fragrant, aroma is persistent, a mix of citrus and menthol. The tea obtained from the flowers of var. Ossa is extremely fragrant and golden-yellow in colour. The average weight of an inflorescence is 0.96 g compared to 2.5 g in var. Domnesc. The average number of inflorescences per bush is 290 and the average flower mass is 278 g. The average number of stems is 192 and the average weight of a stem is 3.69 g, resulting in an average leaf mass of 708 g. The average diameter of an inflorescence in var. Ossa is 1.1 cm compared to 0.9 cm in var. Domnesc, which is due to the large vertices with spaced star-shaped bracts. The first wave of flowering starts at the end of May and flowering is complete in the second week of June. The second wave of flowering lasts throughout October, in long autumn conditions the flowers can stay on the stem until frost.

Crop technology

For optimal development, humus-carbonate soils, similar to those in the area of origin, are recommended. Acid soils destroy the root and stop the germination of the seeds. Development is optimal in the case of limestone amendments. The plant's water needs are provided by the ramifications of the root system, which penetrate deep into the cracks of the soil, conferring resistance to drought, to temperatures up to +45°C but also to extreme minimum temperatures of -20°C, proving ecological plasticity (Vînătoru et al., 2019).

Propagation in *Sideritis scardica* var. Ossa is made by seeds. The optimal planting establishment scheme is 70 cm between rows and 40 cm between plants/row, compared to 50 cm between plants/row in var. Domnesc (Figure 7) The smaller spacing is due to the smaller height and diameter of the plant.

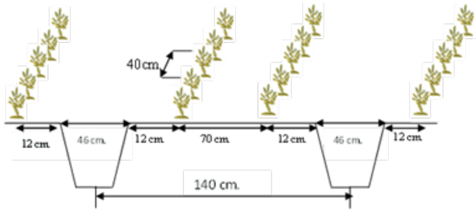

Figure 7. *Sideritis scardica* var. Ossa - scheme culture: 70 cm between rows and 40 cm between plants/row

A similar establishment scheme was used in an experiment of the Genetic Resources Bank in Thessaloniki with the *Sideritis syriaca* species. Planting took place during mid-autumn after the first rains in well-drained soils of the BBGK and the planting density was 0.70-1 m between lines X 0.50-0.60 m on lines (Kloukina et al., 2020).

Regarding the *Sideritis scardica* var. Ossa it can be observed in Table 1 the growth evolution of the G2 genotype in the first three years crop: average plant height between 13-33 cm, canopy diameter range 17-58 cm, with a third year spectacular growth; also, the number of sprouts from 4 to 44; no flowering for the first year, 56 inflorescences/plant in year 2 and 95 in year 3;

converted into vegetative mass were obtained from 114 g/plant in the first year to almost 600 g/plant in the second year and 749 g/plant in the third year crop. After harvesting, the flowering stems are dried in specially designed areas, away from sunlight and draughts; the stems are tied in bunches that weigh about 400 g in green. The plant dehydration percentage was between 70-75%. In the graphic representation (Figure 8) it can observe both the evolution of the vegetative mass and the final results obtained after drying and storage.

Table 1. Biometric measurements regarding vegetative mass in Ossa cultivar

G2 type	Canopy diameter (cm)	Plant height (cm)	Number of sprouts	Number of leaves / sprout	Number of leaves/ plant	Number of inflorescences	Number of flowers stems	Green vegetative mass (g)	Dry vegetative mass (g)
Year1	17.3±0.67[c]	13.10±1.41[bc]	4.4±0.89[a]	4.4±1.21[a]	19.7±5.43[a]	0,03±0.89[c]	0.04±0.55[c]	114±11.10[c]	27.89±4.23[c]
Year2	39.88±1.49[a]	23.46±1.95[b]	37.2±1.3[b]	4.8±1.1[a]	183.5±21.3[b]	56.6±7.13[b]	35.3±0.35[b]	598.3±15.4[b]	161.14±13.21[b]
Year3	71.11±5.47[a]	32.55±2.19[a]	44.6±1.88[a]	6.5±1.12[a]	264.7±35.14[a]	95.4±4.31[a]	79.9±8.98[a]	708.51±8.76[c]	201.9±11.25[a]

*Letters represent Duncan test results with 95% confidence interval and p < 0.05%.

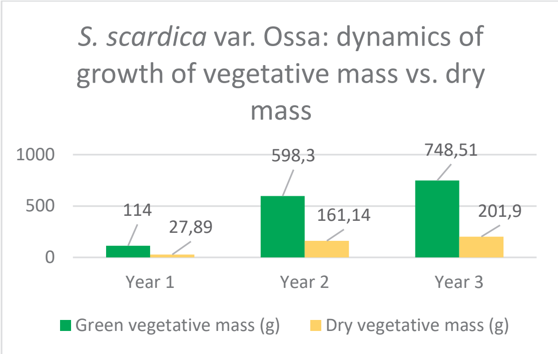

Figure 8. Graphic representation of the evolution of vegetative mass vs. dry mass

Figure 9. *Sideritis scardica* var. Ossa - plant details

Several species of the genus *Sideritis* have been studied for the first time in Romania at PGRB Buzau, such as: *S. scardica*, var. Domnesc, *S. scardica* var. Ossa, *S. syriaca*. *S. hyssopifolia*. This paper is focused on *S. scardica* var. Ossa due to its aromatic and medicinal potential. Breeding and acclimatization of this genotype found only on Mt. Ossa has demonstrated that it shows increased resistance to the climatic conditions of our country, is not a demanding plant and does not require special conditions for care and cultivation. On the contrary, irrigation below normal flow will positively influence the concentration of essential oils. The aerial parts of these plants are used to prepare very aromatic tea. In addition, var. Ossa is also an ornamental and melliferous plant (Figure 9). Being a perennial plant, it is recommended for decorating gardens, especially rockeries, as the silvery-green colouring of the leaves, the yellow herringbone-shaped inflorescences add value to the yard. Its strong aroma, a mix of citrus and menthol, attracts bees all day long because *Sideritis* spp. are highly appreciated as a honey plant, providing pollen and nectar starting early summer until frost. During the two flowering waves, May-June and September-October, the plant is practically completely covered by bees. For this reason, harvest is recommended in the early hours of the morning followed by storing the plants in well-ventilated spaces, protected from sunlight.

CONCLUSIONS

Sideritis scardica var. Ossa was acclimatized by the PGRB Buzau researchers team, obtaining a valuable G2 variety that shows genetic stability in the offspring, as well as valuable phenotypic and biochemical characteristic;

The cultivar can be successfully cultivated in an ecological system, being appreciated as food, ornamental, honey plant, but especially as a medicinal plant due to the active principles in its biochemical composition.

The culture technology for this species was developed. In the future, PGRB Buzau will provide growers with both seeds and seedlings for testing.

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APPLICATION OF SUPERABSORBENT POLYMERS IN THE AGRICULTURE AND THE IMPORTANCE OF THEIR BIODEGRADABILITY - A REVIEW

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Abstract

This article aims to bring to light a relatively new strategy to ameliorate some problems that the agricultural sector faces in the current situation at a global level, such as water scarcity, drought, continuous degenerative processes of the soil or even the diseases caused by plant pests. One of the novel solutions that can be applied to balance the current situation is represented by the using of biodegradable superabsorbent polymers, due to their capacity, representing a reservoir able to store water, nutrients, or pesticides and then release them a constant flow, ensuring an optimal and beneficial ratio at the soil and plant level. They have numerous beneficial properties and advantages, being found in a multitude of fields, one of the most critical properties being represented by their biodegradability. Currently, the most "green" methods are being sought for obtaining these superabsorbents polymers to be used on a large scale and presenting a ratio of biodegradation as high as possible and no ecotoxicity after their application.

Key words*: superabsorbent, biopolymer, biodegradability, hydrogel, water storage.*

INTRODUCTION

In the current situation, the global population faces severe water shortages throughout the year with areas where water resources are limited for at least one month in a year (Kathi et al., 2021).
Agriculture is the sector that uses over 70% of fresh water, therefore, according to Abobatta (2018), in order to feed a population of approximately 9 billion individuals, until the year 2050, a calculable 50% increase in the agricultural production would be required and also 15% of water withdrawals.
Millions of hectares of land are becoming unproductive every year due to the agricultural activities becoming less and less sustainable which results in the triggering of soil degenerative processes (Turioni et al., 2021).
The sandy, calcareous, alkaline and acidic soil are in which restoration is necessary, they have very low productivity and are poor in terms of their biological or physico-chemical properties (Rajakumar & Sankar, 2016).
Drought is one of the most harmful, expensive, and complicated natural disasters that has an impact on many facets of civilization (Craft et al., 2015). Low soil moisture and high temperatures prevent plants from growing properly, which reduces agricultural yield and quality (Skrzypczak et al., 2020). Water stress, particularly during the crucial development phase of the farmed crop, is one of the most significant elements impacting plant growth and agricultural output (Barajas-Ledesma et al., 2022). The most popular irrigation technique involves directly watering the crop surface; however, this doesn't address the drought issue (Skrzypczak et al., 2020). The introduction of contemporary micro-irrigation technologies, such as low-pressure micro-sprinklers and drip irrigation systems, can now alleviate the problem by substantially lowering irrigation water consumption and boosting water usage efficiency (Abd El-Wahed & Ali, 2013; Deshmukh & Hardaha, 2014). Yet, these high-tech procedures are specifically utilized in high-value crops and need substantial capital expenditures, ongoing running expenses, and farmer experience (Patra et al., 2022). The use of hydrogels with strong water absorption and

retention capabilities, even at high temperatures, is one of the temporary solutions to these deficiencies (Skrzypczak et al., 2020). The hydrogel functions as a reservoir, storing and releasing a steady flow of water and nutrients required by plants to thrive (Kurrey et al., 2018). According to the Food and Agriculture Organization of the United Nations (FAO), plant diseases can lead over $220 billion in global economic losses annually, while plant pests contribute to losses of 20-40% of overall crop yield globally (Pirzada et al., 2020). Pesticide delivery methods based on hydrogels have indeed attracted attention, and various patents have been obtained based upon those particular agricultural purposes (Montesano et al., 2015).

The majority of traditional hydrogels on the market are acrylate-based products which aren't biodegradable and are considered potential soil pollutants. However, with the increased focus on environmental protection issues, biodegradable hydrogels are attracting a grown interest for considerable commercial uses in agriculture (Cannazza et al., 2014).

Although most superabsorbents are composed of synthetic polymers (often acrylic monomers) because to their favorable cost-performance ratio, for environmental considerations, the notion of partially or entirely substituting those synthetic materials with sustainable and “green" alternatives should be studied (Michalik & Wandzik, 2020). The ecologically responsible manufacture of biodegradable hydrogels from natural resources is in high demand (Monadal et al., 2022). Among the most important polysaccharides integrated into hydrogels with potential for large-scale agricultural applications are chitosan (Nangia et al., 2018), alginates (Thakur et al., 2018), starch (Ismail et al., 2013), and cellulose (Demetri et al., 2013; Tomadoni et al., 2019).

Numerous natural ingredients have recently been effectively incorporated into hydrogels to increase their biocompatibility and biodegradability, resulting in new biodegradable superabsorbent hydrogels with importance in the agricultural sector, based on starch, natural and semi-synthetic chitosan, cellulose, pectin, gum arabic and cashew gum (Čechmánková et al., 2021).

MECHANISM OF ACTION

Hydrogels, furthermore known as "root watering crystals," "water retention granules," or "raindrops" (Patra et al., 2022), are cross-linked polymers with hydrophilic groups that can absorb a significant quantity of water without being dissolved in it, retaining both water and nutrients and then releasing them over time (Mahinroosta et al., 2018). Because of their cross-linked structure, hydrogels retain the equilibrium of their networks when inflated, enabling them to remain stable in a range of settings. Certain hydrogels may even absorb aqueous fluids up to hundreds of times their weight and are hence referred to as super absorbents (Mignon et al., 2019). Hydrogels are unusual materials due to their softness, intelligence, and the capacity to retain water (Choudhary et al., 2014).

An ideal agriculturally effective hydrogel should meet several characteristics, including “substantial absorption ability, adjustable absorption rate, high absorption when exposed to water, high gel fraction after crosslinking, low cost, high stability after swelling and during storage, non-toxicity, and rewetting capacity” (Michalik & Wandzik, 2020).

The hydrogel is composed of a system of parallel polymer chains which are commonly joined together by cross-linking agents (Narjary et al., 2013).

The cationic hydrogels adhere to clay components and behave as flocculants, whereas anionic hydrogels can form bonds with clay as well as other negatively charged particles via ionic bridges (calcium and magnesium). The superabsobent’s capacity to absorb water, form aggregates and preserve soil structure is directly influenced by the attraction between the polymer and the solutes and soil particles in the environment, as a result, “the more cross-linked the polymer, the tighter the network” (Rajakumar & Sankar, 2016).

CLASSIFICATION

Hydrogels can be classed based on a variety of factors, but the primary criterion for classification is their origin. The two basic varieties are synthetic and natural. Natural hydrogels (collagen, agarose, gelatine) are composed of polysaccharides with the capacity to form a hydrophilic chemical structure with high biocompatibility, whereas synthetic hydrogels (hydrophilic polymers) are created by chemical stabilization of hydrophilic polymeric materials in a three-dimensional network (Skrzypczak et al., 2020).
Hidrogels can be classified using the criteria listed in Table 1.

Table 1. Classification of hydrogels

Criterion of classification	Type of hydrogel	Reference
Origin	- syntetic - natural	Skrzypczak et al., 2020
Physical-chemical properties	- physically stable - chemically stable	Elshafie & Camele, 2021
Method of preparation	- homopolymer - copolymer - semi-interpenetrating networks - interpenetrating networks	Laxmi et al., 2019
Configuration	- amorphous (non-crystalline) - semicrystalline (complex mixture of amorphous and crystalline phases)	Garg & Garg, 2016
Physical appearance	- solid - semisolid - liquid	Varaprasad et al., 2017
Physical properties	- convențional - smart	Bustamante-Torres et al. 2021
Cross-linked chain charge	- nonionic - ionic - amphoteric - zwitter	Khan et al., 2016
Pore size	- non-porous - microporous - super porous	Devi & Gaba, 2019
Degradability	- biodegradable - non-biodegradable	Vinchhi et al. 2021
Response to stimuli	- thermosensitive -sensitive to magnetic and electric field - sensitive to the composition of solvents - sensitive to pH -sensitive to sound and molecular species	Ahmad et al. 2022

The majority of traditional hydrogels on the market are acrylate-based products; the type of crosslinking agent used in chemical polymerization methods strengthens the bonds between the chains; as a result, as compared to their natural equivalents, the compounds are not biodegradable or degrade at a considerably lesser rate, and thus might be regarded a concern to possible soil contamination (Mallik et al., 2018). Given the increased concern about environmental problems, there is a high need for environmentally friendly biodegradable hydrogels that do not cause soil deterioration or phytotoxicity (Skrzypczak et al., 2020). Among natural hydrogels, the polysaccharide hydrogels are particularly of interest because they are biodegradable, have renewable capacity, present low-cost and nontoxicity, and simple to produce from easily accessible precursors (Turioni et al., 2021).

ADVANTAGES AND DISADVANTAGES

The usage of hydrogels in agriculture can provide the following benefits: water conservation, resistance to biotic and abiotic drought stress, improved soil quality, decreased seedling mortality, reduced irrigation frequency and water consumption, and reduced use of fertilizers and pesticides (Guillherme et al., 2015). They prevent soil erosion caused by surface runoff as well as fertilizer/pesticide leakage into groundwater (Sarkar et al., 2017), and also increase soil physical properties by increasing water retention and infiltration capacity, reducing the necessity of continuous watering (Skrzypczak et al., 2020).
Hydrogels offer a wide range of uses, including agriculture, drought control, water saving, industrial planting and municipal gardening (Elshafie and Camele, 2021). Apart from agricultural sector, they have been used in the following areas: biosensor (Ahmed, 2015), antigen delivery systems (Ishii-Mizuno et al., 2017), tissue engineering and regenerative medicine (Wei et al., 2016), controlled drug release (Zhao et al., 2016), therapeutic applications (Schulze et al., 2016), antifouling paints (Wang & Wei, 2016), artificial snow (Ahmed, 2015), biomedical and pharmaceutical applications (Wang et al., 2015), dressing and sealing (Ebrahimi et al., 2015), drug delivery systems (Ye et al., 2016) and water purification (Tran et al., 2017).
Among the general drawbacks of the usage of hydrogels, the most common are the non-

adherent nature, the low mechanical strength, the nutrient/agrochemical loading weight, or the interference of dissolved salts (Rajakuar & Sankar, 2016).

The expensive cost of hydrogels is a deterrent that has severely limited their broad acceptance. (Choudhary et al., 2014).

One of the primary drawbacks of hydrogels is that the majority of them are polyacrylamides (recognized carcinogen that endangers both the environment and human health) generated from fossil fuels, which have limited environmental degradability, yielding acrylate and acrylamide groups in the soil (Barajas-Ledesman et al., 2022).

The water absorption capacity could be limited when salts are present in the solution, causing a deficiency in the balance between the system's osmotic pressure and the hidrogel's expansion rate (Neto et al., 2017).

According to Laxmi et al. (2019), the characteristics of an ideal hydrogel are: "colorless, odorless and non-toxic materials, with high water absorption capacity, very good functionnality even at high temperatures, improve the physical condition of the soil (porosity, bulk density, water holding capacity, permeability), having desired absorption rate according to application requirements, cost-effective, high durability and stability in the swelling environment and during storage, pH-neutrality after swelling in water, photostability, ability to rewetting, high biodegradability without the formation of toxic species following degradation".

PROCESS OF BIODEGRADATION

A biodegradable material has the property of being entirely utilised as a carbon and energy source enabling microbial growth (based on the OECD definition of "ultimate biodegradability"). The degradation process produces carbon dioxide, mineral salts, water, and new biomass under aerobic circumstances. Methane and/or low molecular weight acids may additionally be generated in anaerobic circumstances (Harrison et al., 2018).

Hydrogels are classed as either non-biodegradable or biodegradable (Vinchhi et al. 2021). Non-biodegradable hydrogels (majority of synthetic ones, obtained through chemical cross-linking) are distinguished by their resilience to the impacts of environmental stimuli as well as their capacity to retain their chemical, physical, and structural qualities over time. The great majority of natural polymer hydrogels, on the other hand, may be categorized as biodegradable. When exposed to natural conditions, the three-dimensional structure is prone to deterioration due to enzymatic and bacterial action (Oladosu et al., 2022).

Biodegradability is a highly sought quality in hydrogels intended for agricultural use. Many studies have been undertaken to assess the biodegradability of various hydrogels. Acrylic acid or acrylamide have mostly been used to obtain synthetic polymers, which may represent environmental risks due to their low biodegradability (approximately 10% per year) (Prakash et al., 2021).

According to research, the hydrogel is susceptible to UV radiation and dissolves to form oligomers. Polyacrylate degrades at rates of 10-15% each year into water, carbon dioxide, and nitrogen compounds as it becomes increasingly vulnerable to aerobic and anaerobic microbial breakdown. The polymer's molecules become too large to be absorbed by plant tissue and therefore have no bioaccumulation capability (Rajakuar & Sankar, 2016, Neethu et al., 2018).

Mineralization can also occur as a result of biological degradation, such as that caused by fungi. The biological breakdown of several types of polymers in soil is quite successful, especially under circumstances that significantly enhance solubility. For instance, under aerobic circumstances similar to the breakdown of organic matter in forest regions, the biodegradation of acrylate-based hydrogels in municipal compost has been accomplished at a rate of 1 to 9% per year (Elshafie & Camele, 2021).

Variations in visual appearance, mass, or mechanical qualities take place during different phases of biodegradation, making it critical to determine the moments where such physical changes are best assessed and what appropriate information, they may offer regarding the polymer breakdown. Biodegradation is commonly separated into three stages: "biodeterioration, bio fragmentation, and microbial assimilation". The mechanical properties of the material are commonly changed throughout the biodeterioration stage and the beginning of fragmentation stage. As biodegradation progresses, they are followed by modifications in visual appearance and mass loss, establishing a primal assessment of biodegradation (Harrison et al., 2018).

METHODS FOR ASSESING THE BIODEGRADABILITY OF HYDROGELS

One of the most commonly assessment method of the biodegradability of hydrogels is the ***soil burial method***.

The percentage of biodegradation and the duration of the process could differentiate based on the materials and the method used. Table 2 showcases the compared weight loss percentage of several hydrogels after biodegradation, based upon the materials utilised for the synthesis of the hydrogels and the number of degradation days. As it could be seen, using the soil burial method, most of the hydrogels have a percentage of biodegradation around 80-90% on a 70-80 days trial.

The highest percentage was obtained by Saruchi et al. (2015), 92.29% in 77 days trial on gum tragacanth-acrylic acid-based hydrogel while, Choudhary et al. (2022) obtained for their gellan gum, ammonium persulfate, acrylic acid, N, N′-methylene bisacrylamide based hydrogel, in a 22 days trial, a record of 86.71% biodegradation rate (Table 2).

Taghreed et al. (2022) buried in pots filled with garden soil, their synthesized hydrogels (1g) equidistantly (3 cm apart). The weight of each sample was determined by extracting the samples every 7 days, rinsing with water, and drying at 70°C. Visual evaluation, changes in morphology (SEM), chemical structure (FTIR), and weight loss (WL) analyses can attributed to measure the level of degradation at various phases of biodegradation:

The weight loss (WL) of the samples could be obtained using the following equasion: “WL (%) = Wi − Wf/ Wi × 100” where Wi represents the initial weight of the samples before the biodegradation process, while Wf represents the weight of the samples after specified time intervals of biodegradation (Durpekova et al., 2021).

The rate of deterioration, according to Choudhary et al. (2022), is highly reliant on various parameters that impact microbial proliferation, such as pH, oxygen concentration, temperature, mineral nutrition supply, and moisture. The percentage weight loss rises with time, which might be caused by residual or low molecular weight macromers dissociating during the testing.

Kenawy et al. (2021) assessed biodegradability by monitoring the weight loss of soil samples over time. Dried gel samples were weighed (1 g) and then buried in soil (maintained at 20% moisture) at a depth of 15 cm for 150 days. The buried samples were examined at certain time intervals, washed with distilled water, dried under vacuum at 60 ± 2°C for 24 h, and then conditioned in a desiccator for at least one day. A continuous weight loss of the sample was observed over time and after 160 days a total weight loss of about 60% was reached showing good degradability in soil.

Choudhary et al. (2020) obtained by microwave -assisted synthesis, a new biodegradable superabsorbent Agr/GE-co-MA/AA (agar-agar gelatin copolymerized methyl acrylate and acrylic acid hydrogel) developed as an effective water retention agent. They determined that the degradation of Agr/GE-co-MA/AA hydrogel in soil and sand is a direct result of the activity of microorganisms, having no harmful effects on the fertility of sand and soil and improving organic matter in the agricultural field.

Another common method of determining the biodegradability of hydrogels is through ***composting***.

Composting is an aerobic technique that manages solid waste, in which biodegradable materials are biologically digested into humus (a valuable nutrition source for increasing soil fertility and agricultural production) in the presence of microorganisms under supervised conditions such as temperature, aeration and humidity (Pires et al., 2022).

Sharma et al. (2014) used the compost collected from the municipality sewerage plant in Solan, Himachal Pradesh, India. The hydrogel samples of almost equal weights were buried in pots in the compost at the depth of 10 cm. Each day the compost in the pots was fed with microbial concentrate collected from the sewerage plant site and the biodegradability of the hydrogels was measured for 60 days up to specific time interval. The samples were taken out, washed with distilled water and dried in vacuum oven at

60°C followed by the weighing process. After 60 days, the hydrogels tested deteriorated at a rate of 1.07 mg/day for the Gg-cl-poly(AA) hydrogel and 90% at a rate of 1.5 mg/day for the Gg-cl-poly(AA-IPN-aniline) hydrogel.

Table 2. Comparison of the biodegradation of hydrogels using the soil burial method

Hydrogel	Materials	Percentage of Degradation	Total days	Reference
Gum tragacanth-acrylic acid based hydrogel	Gum tragacanth, Acrylic acid	92.29	77	Saruchi et al., 2015
L/KJ/SA hydrogel	Lignosulfonate, Sodium alginate, Konjaku flour	20	120	Song et al., 2020
Lipase catalyzed hydrogel-IPN of GT with poly(AAm) and poly(MAA)	Gum tragacanth, Acrylamide, Methacrylic acid	81.26	77	Saruchi et al., 2019
CS50 hydrogel	Cassava starch, Polyacrylamide	80	30	Junlapong et al., 2020
Xanthan gum based hydrogel (semi-IPN)	Xanthan gum, Polyacrylic acid	78.3	70	Sukriti et al., 2017
GG-cl-poly(AA)	Gellan gum, Ammonium persulfate, Acrylic acid, N, N′-methylene bisacrylamide	86.71	22	Choudhary et al., 2022
Av-cl-poly(AA)	Aloe vera powder, Acrylic acid, Glutaraldehyde, Ammonium persulfate	90	70	Saruchi et al., 2023
CAP hydrogel film	Chitosan, Acetic acid, Acrylonitrile, Polyol, Bisacrylamide	90	42	Kouser et al., 2018
Neem gum–grafted poly (acrylamide) hydrogel	Neem gum polysaccharide, Acrylamide	90	28	Malviya et al., 2019
PVA/Water Hyacinth (PW) hydrogel	Polyvinyl alcohol, Water-hyacinth	12.86	42	Hossain et al., 2021
Gum rosin and psyllium-based hydrogel	Gum rosin, Psyllium	86.8	63	Wadhera et al., 2020
Enzymatically catalyzed IPN	Lipase as initiator, Glutaraldehyde, Acrylic acid, Acrylamide	86.03	77	Saruchi et al., 2016
CMC/P4VP hydrogel	Carboxymethyl cellulose, Poly (4-vinylpyridine), N, N- methylene bis-acrylamide	50	5	Mohamed et al., 2022

Vermicomposting is another used method to determine the biodegradability of hydrogels.

Vermicompost is the end result of a biodegradation involving a wide range of worm species, most notably red worms, white worms, and various other earthworms and, according to the study by Choudhary et al. (2022) biodegradation by soil burial test resulted in lower degree of less weight loss than vermicomposting. This could occur based on the fact that the vermicompost contains more types of microorganisms than regular garden soil, which accelerates the breakdown process. Because of its high NPK content, as well as its capacity to strengthen the structure of the soil and store more water, vermicompost has a higher breakdown rate.

In the study assessed by Bandyopadhyay et al. (2019), the hydrogel film showcased an 80% rate of degradability in 28 days using the vermicomposting method.

Saruchi et al. (2023) assessed the biodegradability of a newly synthesized Av-cl-poly (AA) hydrogel over a period of 70 days using all three of the methods. The percentage of biodegradation in the study has proven to be 90% soil burial method, 93% vermicomposting and 94% composting.

The study of biodegradability with the white fungus of the polyacrylate and polyacrylamide copolymer in the soil along with the soil microbiome was explored. It demonstrated that microbial degradation was significantly lower than degradation paired with fungus or bacteria. The deterioration, however, was adequate for certification. In an open environment degradation research, it was conclusively demonstrated that microbial activity was responsible for the deterioration of the polymer-based hydrogel (Prakash et al., 2021).

Turioni et al. (2021) investigated the degradation of hydrogels using home-made devices that allowed indirect interaction between hydrogels and soil. Deionized water enriched with chemicals/microorganisms from the soil layer was deposited in the top tube to assure hydration, the hydrogel was placed in a cell created between two Falcon tubes in contact with two synthetic fabric discs. A rubber band linked the two tubes together, which was kept in place by two more rubber bands. This configuration allowed the operator to open the system and enter the cell containing the hydrogels.

There are a variety of analytical techniques and methodologies that may be used to supplement physical testing methods for determining biodegradability, including scanning electron microscopy - SEM (Kolya & Kang, 2022) transmission electron microscopy - TEM (Shaghaleh et al., 2022), thermogravimetric analysis - TGA (Songara & Patel, 2021) and Fourier transform infrared spectroscopy - FT-IR (Bora & Karak, 2022).

CONCLUSIONS

The purpose of sustainable agriculture is to improve output while inflicting as little environmental damage as possible. Hydrogels might be used to alleviate drought stress and increased soil degradation, both of which represent a serious danger to agriculture. Although various studies regarding the application of hydrogels in agriculture showcase their tremendous value, the cost and potential toxicity from biodegradation constitute issues that must be addressed during the following decade by using hydrogels that use non-toxic materials, possess elevated capability to absorb water, excellent functionality even at high temperatures, and, most importantly, high biodegradability without the generation of harmful compounds.

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NATURA 2000 HABITATS FROM OLTENIA AFFECTED BY INVASIVE AND POTENTIALLY INVASIVE SPECIES (I)

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Abstract

The present paper analyzes some forest habitats from Oltenia, which are affected by different invasive and potentially invasive species. The large number of habitats present on the territory of Oltenia did not allow their unitary research. In this paper, references are made only to the habitats 91M0, 91E0, 91Y0, and 92A0. The main factor that greatly contributed to the introduction and rapid spread of these invasive and potentially invasive plants in the analyzed habitats was zoo-anthropogenic. The changes that occurred affected both the structure and the functions of these habitats. Among the invasive and potentially invasive species identified in the studied habitats, which affect their floristic composition, we mention: Ambrosia artemisiifolia L., Acer negundo L., Artemisia annua L., Asclepias syriaca L., Bidens frondosus L., Echinocystis lobata (Michx) Tor. et A. Gray, Prunus cerasifera Ehrh., Robinia pseudoacacia L., and Xanthium orientale L. subsp. italicum (Moretti) Greuter.*

Key words: *habitat, invasive, Natura 2000, Oltenia, Romania.*

INTRODUCTION

Habitats represent a key element of biodiversity, resulting from the complex interaction of biotic and abiotic factors (Gigante et al., 2016). According to some researchers, they are important indicators of biodiversity (Bunce et al., 2013) and their intermediate position between biodiversity levels grants them an important role in the assessment of the state of nature conservation (Gigante et al., 2016). Recent studies on biodiversity protection propose the conservation of habitats, of the surfaces on which they are present, and not the analysis of a single species from an idio-taxonomic point of view (Noss, 1996; Cowling et al., 2004; Nicholson et al., 2009; Berg et al., 2014; Keith et al., 2015).

In accordance with Annex I of the *Habitats Directive* (92/43/EEC), based on their distribution area, the forests under study belong to the main category designated as "Forests of Temperate Europe" and characterized by code 91. Romanian forests are also subject to European law, such as the *Habitats* and *Birds Directives*. However, there is a severe lack of enforcement at all administration levels, even in National Parks (Reif et al., 2022).

In the absence of the negative influences exerted by the zoo-anthropogenic factor on forest ecosystems, they would occupy a significant part of Romania's territory (Giurescu, 1975; Biriş, 2017). Almost half of the forests with high conservation value are under the influence of anthropogenic stress (Munteanu et al., 2021).

Climate variability and change have an impact on crop productivity globally. They also have an indirect impact on biotic constraints, which may lead to the invasion of weeds, pests, and pathogens in previously unaffected areas (Velea et al., 2021; Cotuna et al., 2022a; Cotuna et al., 2022b; Cotuna et al., 2022c).

Thus, worldwide, in the context of climate change, one of the primary concerns in the twenty-first century is agricultural production and food security, especially in vulnerable places (Paraschivu et al., 2022).

Although in Romania, the deciduous forests edified by species of *Quercus* genus are a center of taxonomic and genetic diversity (Neophytou, 2014), the climate changes of recent years have led to modifications in the floristic composition,

quality and integrity of the habitats edified by these species in Oltenia.
There are large unforested areas in the plain regions and in the Piedmont hills, where the habitats analyzed in the present paper find their range. The deforestations were conducted in order to make way for meadows that were used as pastures and for agricultural lands that provided food for the local people.
The analyzed habitats were characterized according to the Manual for Interpretation of EU Habitats in Romania (Gafta & Mountford, 2008), in relation to the vegetation associated with them.
The forests included in habitats 91M0, and 91Y0 are present in small areas, as compared to other forest habitats in Romania. Those belonging to the 91M0 habitat are located in xeric, thermophilic places, while the forests classified as 91Y0 are established on the northern hill slopes. Along with *Quercus cerris* and *Q. frainetto*, in some areas there are also found *Q. polycarpa*, *A. tataricum* and *A. campestre* (Pop, 1942, 1945; Coldea & Pop 1996; Doniță et al., 2008).
The azonal forests included in habitats 91E0* and 92A0 are characteristic from the viewpoint of flora and ecological conditions. They are located in the Danube alluvial plain and along the lower courses of large streams and rivers. The 92A0 habitat has a better representation in the Danube alluvial plain, while the 91E0* habitat is much more widespread in the alluvial plains along the rivers that cross the Piedmont and Subcarpathian hills of Oltenia. The 92A0 habitat consists mainly of *Salix alba*, *S. fragilis*, *Populus alba* (Oprea, 2004), while 91E0* is mostly composed of *Alnus glutinosa*, *A. incana*, and *Fraxinus excelsio* (Gafta & Mountford, 2008).

MATERIALS AND METHODS

Oltenia represents an amphitheater characterized by a great variability of soils (Ionuș et al., 2015) on which different types of vegetation develop. In the context of the general southern exposure of the relief, continental air masses induce a warmer climate (Ivan et al., 1992). Due to these climatic conditions, most of the thermophilous, southern elements are concentrated especially in the southwestern part of Oltenia. All the vegetation zones and levels in Romania can be also found in the region of Oltenia; from the geographical viewpoint, the area belongs to the Oltenia Plain (as part of the Romanian Plain), the Getic Piedmont, and the Getic Subcarpathians (Figure 1).

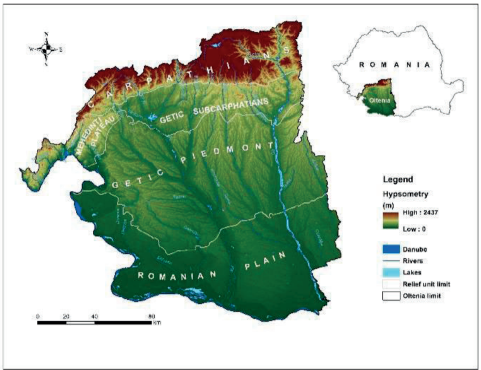

Figure 1. Map of Oltenia

The habitats under study are characterized by certain types of vegetation. Some of these prefer thermophilous, xeric places (e.g. habitat 91M0), while others are located in the alluvial plains of the main rivers (e.g. habitats 91E0* and 92A0), or at the level of the Piedmont hills (e.g. habitat 91Y0).
The present research was conducted on areas where the habitats under study have a favorable conservation status, such as those located in protected natural areas (e.g. "Silvostepa Oltenei" - Dolj County, "Prunișor" - Mehedinți County, etc.), as well as on areas identified in different parts of Oltenia, which are either privately owned or state-owned.
Our research was based on numerous field trips carried out in different stages of vegetation, in order to have a complete understanding of the areas occupied by the habitats under study. Currently, the technology of aerial photography and the use of drones offer the possibility of collecting data and carrying out activities from a reasonable distance, without affecting the areas occupied by forest vegetation (Călina et al., 2020). Their comparative analysis enabled the authors to obtain certain differences in terms of floristic composition.
Increased attention was paid to the species that are on the list of invasive and potentially invasive plants in Romania (Anastasiu et al., 2019), because they induce major imbalances in the ecosystems where they settle in a short period of time. This category of plants continues

to expand the area they occupy at an alert pace. It settles in almost all types of spontaneous vegetation in Romania, especially in those located in the plain region and up to the Subcarpathian hills. Mountain and alpine regions are not affected by these alien invasive species.

Some areas where certain habitats are located (91E0* and 92A0) are prone to flooding (Ionuș et al., 2015), this type of event inducing major changes in the structure and functions of the phytocoenoses located in those places.

The scientific names of the plants identified in the analyzed habitats are in accordance with EURO+MED 2006-.

RESULTS AND DISCUSSIONS

Currently, the areas occupied by forests in Romania (including Oltenia) are much smaller, accounting only for 30% of the country's surface (Forest Europe, 2020).

The reduction of the surfaces was induced by the increased accessibility of people and domestic animals to these ecosystems, as well as by climate changes characterized by increasingly intense manifestations during recent years, such as high temperatures, very low precipitation (almost absent for a rather long period), especially in Oltenia.

All these factors led to obvious changes in the floristic composition of the analyzed habitats, especially through the appearance of numerous invasive and potentially invasive species. Some of these are found in a few specimens (e.g. *Abutilon theophrasti* Medik., *Cytisus scoparius* (L.) Link., *Oenothera biennis* L., *Galinsoga parviflora* Cav. or *Matricaria discoidea* DC.), while others go through a luxuriant development in a short time interval (e.g. *Ambrosia artemisiifolia* L., *Xanthium orientale* subsp. *italicum* (Moretti) Greuter, *Erigeron canadensis* L., etc.).

The 91M0 habitat comprises the xero-mesophilous - thermophilous oak forests that are present in Oltenia, whether on flat surfaces or on slopes with southern, eastern or western exposures. They grow on soils with seasonal humidity fluctuations (excessively wet in spring and dry in summer) (Răduțoiu, 2008). They are well represented in the plain region and in the Piedmont hills, where they occupy the largest areas. In some regions of Oltenia, there were identified forests belonging to this habitat, where uncontrolled clearings are practiced; in other areas, young regenerating forests were found.

By analyzing the floristic composition of the areas where the influence of the zoo-anthropogenic factor is greater, the authors identified the presence of some invasive and potentially invasive species that are favored by human activities. Among these, we mention: *Acer negundo* L., *Ailanthus altissima* (Mill.) Swingle, *Ambrosia artemisiifolia* L., *Erigeron annuus* (L.) Pers. subsp. *annuus*, *Gleditsia triacanthos* L., *Morus alba* L., *Phytolacca americana* L., *Prunus cerasifera* Ehrh. and *Robinia pseudoacacia* L.

The sessile oak forests included in the 91Y0 habitat are located on the upper edge of the forests comprised in the 91M0 habitat, or on the northern slopes of the Piedmont hills. They have a good representation in Oltenia, in the upper part of the Getic Piedmont and in the Subcarpathian hills.

Unlike the 91M0 habitat, the sessile oak forests included in the 91Y0 habitat comprise fewer species from the category of invasive and potentially invasive alien plants.

In some areas of Oltenia, the 91Y0 habitat includes a mixture of species that cannot be assigned to a certain type of vegetation mentioned in the specialized literature for this habitat in Romania.

Among the invasive and potentially invasive plants identified in the areas occupied by the 91Y0 habitat, there are to be mentioned: *Acer negundo* L., *Ambrosia artemisiifolia* L., *Erigeron canadensis* L., *Erigeron annuus* (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz, *Gleditsia triacanthos* L., *Phytolacca americana* L., *Veronica persica* Poir.

The analysis of invasive and potentially invasive allogenic plants from the researched habitats that are located on the territory of Oltenia, with a special view to their population typology, enabled the authors to achieve a classification concerning the aggressiveness shown by these plants (Table 1). It can be noticed that the forests included in habitats 91M0 and 91Y0 have few invasive species, but some of them show high aggressiveness on certain surfaces: *Robinia pseudacacia* L., *Acer negundo* L., as well as *Ailanthus altissima* (Mill.) Swingle in the southwestern part of Oltenia.

Table 1. Invasive and potentially invasive plants from the analyzed habitats

Scientific name	Population typology				Type of introduction
	Habitat 91M0	Habitat 91Y0	Habitat 91E0*	Habitat 92A0	
Robinia pseudoacacia L.	5	5	1	1	accidental
Acer negundo L.	3	2	1	-	accidental
Ailanthus altissima (Mill.) Swingle	4	-	1	3	accidental
Ambrosia artemisiifolia L.	1	1	4	4	accidental
Morus alba L.	1	1	2	4	accidental
Amorpha fruticosa L.	-	-	2	5	accidental
Amaranthus powellii S.Watson	1	-	1	1	accidental
Erigeron annuus (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz	2	1	2	2	accidental
Asclepias syriaca L.	-	-	1	5	accidental
Armoracia rusticana P.Gaertn., B.Mey. et Scherb.	-	-	1	1	accidental
Artemisia annua L.	-	-	1	2	accidental
Galinsoga parviflora Cav.	-	-	2	1	accidental
Erigeron canadensis L.	1	1	2	2	accidental
Cytisus scoparius (L.) Link.	1	-	-	-	accidental
Abutilon theophrasti Medik.	-	-	1	1	accidental
Echinocystis lobata (Michx) Torr. et A. Gray	-	-	1	5	accidental
Veronica persica Poir.	1	1	2	2	accidental
Datura stramonium L.	-	-	1	2	accidental
Xanthium orientale L. subsp. *italicum* (Moretti) Greuter	-	-	3	5	accidental
Sorghum halepense (L.) Pers.	-	-	1	1	accidental
Bidens frondosus L.	-	-	4	5	accidental
Elaeagnus angustifolia L.	1	-	-	1	accidental
Gleditsia triacanthos L.	2	1	1	1	accidental
Helianthus tuberosus L.	-	-	1	4	accidental
Phytolacca americana L.	1	1	4	3	accidental
Bassia scoparia (L.) A. J. Scott	-	-	-	1	accidental
Lycium barbarum L.	1	-	2	2	ornamental
Oenothera biennis L.	-	-	1	1	accidental
Oxalis dillenii Jacq.	-	-	2	2	accidental
Oxalis corniculata L.	-	-	1	1	accidental
Populus × canadensis Moench	-	-	-	1	accidental
Prunus cerasifera Ehrh.	1	1	1	1	accidental
Quercus rubra L.	1	-	-	-	ornamental
Reynoutria japonica Houtt.	-	-	1	1	accidental
Solidago canadensis L.	-	-	2	2	accidental
Xanthium spinosum L.	-	-	1	1	accidental

Population typology: 1- solitary individuals; 2- rare populations, on surfaces < 10 m^2; 3- rare populations, on surfaces > 10 m^2; 4- dense populations, on surfaces < 10 m^2; 5- dense populations, on surfaces > 10 m^2.

Among the researched habitats, those classified as 91E0* and 92A0 have the most invasive and potentially invasive species. The shrub and grass layers are severely affected because of these invading species that form real impenetrable jungles. Such situations were observed in habitat 92A0, developed in the Danube Alluvial Plain and in the lower sector of the Jiu Alluvial Plain, where *Asclepias syriaca* L., *Amorpha fruticosa* L., *Echinocystis lobata* (Michx) Torr. et A. Gray și *Xanthium orientale* subsp. *italicum* (Moretti) Greuter have become invasive on some surfaces (Figures 1, 2, 3, 4) and eliminated almost all species characteristic of this habitat.

In the case of habitats 91M0 and 91Y0, the species that affect their structure and functions are perennial (e.g. *Acer negundo*, *Ailanthus altissima*, and *Robinia pseudacacia*), while in the case of habitats 91E0* and 92A0, the annual species have a good representation on certain surfaces, inducing major changes in the floristic composition of these habitats (e.g. *Asclepias syriaca*, *Echinocystis lobata*, and *Xanthium orientale* subsp. *Italicum*).

In certain habitats (e.g. 92A0), natural and man-induced hazards have led to the appearance of dangerous invasive species that are on the Invasive Alien Species of Concern for the European Union (e.g. *Asclepias syriaca*). Thus, to improve the management of dangerous phenomena represents a key requirement (Licurici et al., 2011).

Figure 1. *Asclepias syriaca* in the Jiu Alluvial Plain, near the confluence with the Danube

Figure 2. *Amorpha fruticosa* in the habitat 92A0 from the Danube Alluvial Plain

Figure 3. *Echinocystis lobata* invasive in the Danube Alluvial Plain

Figure 4. *Xanthium orientale* subsp. *italicum* from the habitat 92A0 located in the Jiu Alluvial Plain

CONCLUSIONS

The forests edified by Quercus species, which are included in the habitats 91M0 and 91Y0 and are located in Oltenia, still represent a precious archive of biodiversity. Therefore, it is necessary to take urgent measures in order to limit the influence of the human factor, which represents the main responsible for the decrease of these areas occupied by forest vegetation and for the alarming expansion of invasive and potentially invasive species.

Among the four forest habitats analyzed in the present paper, those located along the Danube and the main rivers and streams within Oltenia (i.e. 91E0* and 92A0) are the most affected, either by internal grazing, or by overexploitation or dumping of household waste.

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POLYCARPON TETRAPHYLLUM IN ROMANIAN FLORA

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Abstract

This paper brings additional information about the red listed species Polycarpon tetraphyllum from the spontaneous flora of Romania. The research on this taxon started after the identification of a plant material collected from ruderal places of the municipality of Craiova (Dolj County). Polycarpon tetraphyllum is the only species belonging to the genus Polycarpon (Family Caryophyllaceae) that is spread on the Romanian territory. At European level, two species are known (P. polycarpoides, P. tetraphyllum with three subspecies: P. tetraphyllum sp. tetraphyllum; ssp. diphyllum; ssp. alsinifolium). In some European countries, including Romania, this plant is considered to be adventive. The plant analyzed by us in this paper is an Atlantic - Mediterranean taxon, located at the northern limit of the range, found in southern and eastern Europe. In Romania, it is known only from Mehedinți County (i.e. from the localities of Orșova and Vârciorova) and from Dolj County (Valea Stanciului and Craiova). Our research on this species focused on information related to chorology (literature, herbarium and field data), habitat and the phytosociological context in which it was mentioned in the literature and in which it was found in the last two years.

***Key words**: chorology, critically endangered, spontaneous flora, Romania.*

INTRODUCTION

The critically endangered species in Romania (category to which the taxon analyzed by the authors of the present paper also belongs) have drawn the attention of many specialists.

The genus *Polycarpon* L. comprises about thirty-six species that are present in the temperate and warm areas of the two hemispheres (Beldie & Váczy, 1976).

According to *Flora Europaea* (Chater, 1964), four species are known [*P. polycarpoides* (Biv.) Zodda, *P. tetraphyllum* (L.) L., *P. diphyllum* Cav. and *P. alsinifolium* (Biv.) DC.]. The first of them is perennial, while the other three are annual. The second edition of this scientific work (Chater & Akeroyd, 1993) treats the annual species as subspecies of *P. tetraphyllum* [*P. tetraphyllum* (L.) L. ssp. *tetraphyllum*; ssp. *diphyllum* (Cav.) O. Bolòs & Font Quer; ssp. *alsinifolium* (Biv.) Ball] and it is this version that the authors of this paper also accept.

In the flora of Romania, this genus is represented by the taxon ***Polycarpon tetraphyllum*** (L.) L. *Syst. Nat.* ed. 10.2: 881 (1759) subsp. ***tetraphyllum*** (Family Caryophyllaceae) (Chater, 1964, Beldie & Vaczy 1976). Its basionym is *Mollugo tetraphylla* L., Sp. Pl. 1: 89 (1753). It is a native taxon of the Atlantic-Circum-Mediterranean area and it is spread from Europe to Sri Lanka, Macaronesia, northern and northeastern tropical Africa, and the Arabian Peninsula (Figure 1) (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77224385-1#distributions).

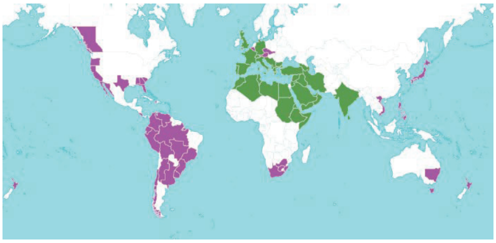

Figure 1. Distribution of the species *Polycarpon tetraphyllum*; green - native; purple - introduced (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77224385-1#distributions)

Polycarpon tetraphyllum (L.) L. subsp. *tetraphyllum* is an Atlantic-Mediterranean taxon

(Jalas & Suominen, 1983), with a northern distribution limit in Romania (Dihoru & Negrean, 2009), widespread in southern and western Europe, while in Belgium and the former Czechoslovakia it is found as an adventitious plant (Chater & Akeroyd, 1993). According to DAISIE, it is also adventive in other European countries (Austria, Czech Republic, Germany, Hungary, Holland, Slovakia, Slovenia, Romania, etc.). It was identified for the first time in the spontaneous flora of Romania by the botanist Iuliu Morariu and it was on that occasion that it also appeared in the specialized literature (Morariu, 1963). Subsequently, it is to be found in different field guides for the identification of spontaneous flora in Romania (Beldie, 1977; Ciocârlan, 2000; 2009; Sârbu et al., 2013). In the Romanian specialized literature, it is mentioned as endangered (Oprea, 2005), critically endangered (Oltean et al., 1994; Dihoru & Negrean, 2009; Negrean 2011; Schneider-Binder, 2014), possibly even extinct (Dihoru & Dihoru, 1994; Dihoru & Negrean, 2009). It is also mentioned from Vârciorova and from the former Ada-Kaleh island on the Danube (Roman, 1974), as well as in the Porțile de Fier [Iron Gates] region, but without specifying the settlement (Roman, 1972, in: Milcu et al., 1972). In Oltenia, it was identified in Craiova Municipality (Cârţu & Hulungă, 1987; Cârţu & Hulungă, 1989) and in Valea Stanciului settlement, both located in Dolj county (Răduţoiu & Costache, 2012).

MATERIALS AND METHODS

The present research started from the identification of a plant material in Dolj County, in ruderal places, among the spaces left by the stones used to pave the alleys.

The authors carried out a follow-up of the adaptation of this species to the conditions imposed by the places where it grows (high temperature, dryness both in the soil and in the atmosphere, pollution, noise stress, etc.).

A few specimens were collected in order to conduct a correct identification based on the botanical literature. Most of the identified specimens were also photographed. Some of them are also inserted in the present work.

The determination of the collected material was carried out using the specialized Romanian and foreign literature (Beldie & Váczy 1976; Chater 1964; Chater & Akeroyd 1993; Beldie 1977; Chater & Akeroyd 1993; Ciocârlan, 2000; 2009; Dihoru & Negrean, 2009; Sârbu et al., 2013). The voucher specimens were stored in the herbarium of the University of Craiova (CRA) (Figure 2) and in that of the "Alexandru Ioan Cuza" University of Iași (I). The authors consulted the main herbaria in the country: Iași ("Alexandru Ioan Cuza" University Herbarium - I), Cluj-Napoca ("Babeş-Bolyai" University Herbarium in Cluj-Napoca - CL), Bucharest (Herbarium of the Institute of Biology of the Romanian Academy - BUCA; Herbarium of the University of Agricultural Sciences and Veterinary Medicine in Bucharest - BUCAG, Herbarium of the "D. Brândză" Botanical Garden in Bucharest - BUC), Craiova (Herbarium of the University of Craiova - CRA), Galați (Herbarium of the Galați Museum of Natural Sciences - GLHM), Timișoara (Herbarium of the University of Timișoara TIM). The acronyms of consulted herbaria are in accordance with Index Herbariorum (Thiers, 2022+).

Figure 2. Herborized plant material with *Polycarpon tetraphyllum*, included in the CRA herbarium

RESULTS AND DISCUSSIONS

The comparative analysis of data collected from the specialized literature, from Romanian herbaria and from personal observations in the field enabled the authors to obtain information that contributes to the enhancement of chorological and phenological data related to the taxon *Polycarpon tetraphyllum* ssp. *tetraphyllum* from Romania.

Short history. In the specialized literature, this species is mentioned for the first time by Schur (1866) in Transylvania (near Cluj, in the Transylvanian Plain), this information being in accordance with Lerchenfeld's herbarium, 1870 (data disputed by Simonkai 1886) (Sîrbu & Oprea, 2011). D. Mititelu collected the species for the first time and deposited it in a public herbarium; the respective herbarium specimen was collected from Orșova (Mehedinți county) on July 20, 1962 (herbarium I, sheet no. 186660). However, the first publication in which the species *Polycarpon tetraphyllum* from Romania appears is the one elaborated by Morariu, who mentions the species from Orșova, Mehedinți county (Morariu, 1963; Beldie & Vaczy, 1976). In this respect, evidence is given by the herbarium sheets with this plant species collected by the author, stored in the herbarium of Cluj-Napoca (CL), or collected by D. Mititelu (1962) and deposited in the herbarium of the "Alexandru Ioan Cuza" University in Iași (I). In Orșova (Mehedinți County), the species grows in uncultivated, ruderal, sunny places, among trees and on the side of the streets (Morariu, 1963; Ciocârlan 2000; 2009). In the Romanian herbaria, there are herbarium sheets with this plant also collected by: Vicol & Schneider (1968) and Roman (1968) from Ada-Kaleh Island (material existing in the herbarium of "Babeş-Bolyai" University in Cluj-Napoca (CL) and, respecttively, in the herbarium of the "D. Brândză" Botanical Garden in Bucharest - BUC (Popescu, 1968) and from Orșova (material existing in the BUCA herbarium), as well as by Costache and Răduțoiu (2010) from Valea Stanciului settlement in Dolj county.

In recent times, it has been also identified as follows:

- near the Mraconia Monastery, Mehedinți County, on the left bank of the Danube, on the side of the Orşova - Baziaş road (August, 2020, leg. Adrian Oprea, personal herbarium);
- in Calafat (leg. Dino Marchetti, personal communication, 2017);
- in Craiova, in the "Nisipuri" cemetery (Cârţu & Hulungă, 1987; Cîrțu & Cârțu, 1989; Negrean, 2011).

Taxonomy. *Polycarpon tetraphyllum* ssp. *tetraphyllum* is a taxon that differs from the other subspecies, irrespectively ssp. *diphyllum* and spp. *alsinifolium* taxa present in the flora of Europe through the following characteristics: leaves without purple shades, lax inflorescence, with visible ramifications and sepals less than 2 mm long.

The main discriminating features found in the material collected by us are the following: short plant, with a strongly branched stem (Figure 3) on which there are obovate leaves, usually four at a node. The inflorescence is loose, strongly branched, with visible branches. The flowers are small, with petals shorter than the sepals, hyaline and emarginate.

Figure 3. Physiognom/habitus of *Polycarpon tetraphyllum* in the areas where it was identified

Biology. It is an annual-perennial plant, hexaploid, 2n=54 (Ciocârlan, 2000), which blooms from June to September. During fieldwork, we noticed that along with the climate changes, the anthesis period has also changed; thus, in the field, we also found flowering specimens in November.

Habitat and coenology

It is a species that prefers anthropophilic habitats or those where humans exert a strong influence on their floristic composition. The analysis of the areas where this plant was identified leads to the conclusion that it is a heliophilous, thermophile taxon, which does not tolerate competition with other species and, therefore, it prefers ruderal places or those with different degrees of anthropization (Figure 4).

During the first year of observation (2021), the species was growing alone or accompanied by *Arenaria serpyllifolia* L., *Sagina procumbens* L.

and *Bryum argenteum* Hedw. and during the second year, the authors found that the analyzed plant developed very well, expanding its range. In the places where it was identified, the plant grows alongside a few species: *Arenaria serpyllifolia* L., *Stellaria media* L., *Polygonum aviculare* L., *Eragrostis minor* Host, *Bryum argenteum* Hedw., *Veronica hederifolia* L., *V. persica* Poir., *Sagina procumbens* L., *Euphorbia maculata* L.

Figure 4. Habitat of the species *Polycarpon tetraphyllum*

In most phytosociological studies on the vegetation, this taxon is mentioned as belonging to the class *Plantaginetea majoris* Tx. et Prsg. 1950 (Dihoru & Negrean, 2009; Sârbu et al., 2013; Schneider-Binder, 2014; Sanda et al., 2003). The analysis of the places where this species was observed and studied justifies our opinion that the vegetation of these surfaces evolves towards the association *Sagino-Bryetum argentei* Diemont, Siss. et Westhoff 1940.

Chorology. The distribution maps of the species were obtained by using the *Corolog 2010* program, realized in the Institute of Biology Bucharest and authored by Sorin Ştefănut. The program uses an Access database, with information originating in the specialized literature, in herbaria and in the field, as well as two types of maps, i.e. the map of average annual temperatures in Romania (Figure 5) and the map of average annual precipitation in Romania (Figure 6).

CONCLUSIONS

Along with a brief history, taxonomy, biology, habitat and coenology information, the paper also presents chorological data originating in the botanical literature, herbarium collections and personal observations in the field, which are also represented on two maps that show correlations with average annual temperatures and precipitation amounts. Thus, the present paper contributes to a better understanding of the chorology and phenology of the species.

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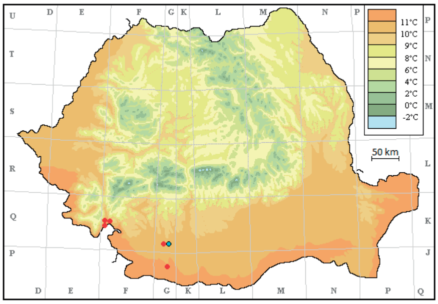

Figure 5. Distribution of the species *Polycarpon tetraphyllum* in Romania, correlated with the average annual temperatures

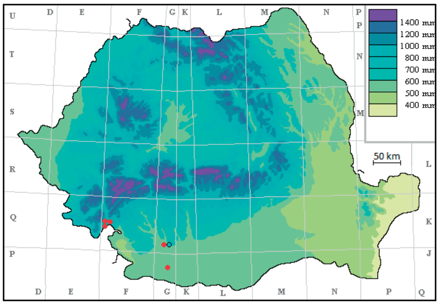

Figure 6. Distribution of the species *Polycarpon tetraphyllum* in Romania, correlated with the average annual precipitation

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BY-PRODUCTS FROM THE ESSENTIAL OIL INDUSTRY - VALORIZATION AND RECYCLING. PRACTICAL APPLICATION - ADSORPTION OF TEXTILE INDUSTRY DYES

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Abstract

The purpose of this study is to enrich the approaches and methods for valorization and utilization of the essential oil industry by-products and to outline the perspectives in this area. As a possible practical application lavender and rose by-products were used as a bio adsorbents for removal of textile industry dyes. The influence of contact time, adsorbent amount and temperature was investigated. The efficiency of adsorption with lavender and rose by-products was compared with activated carbon, Al_2O_3 and silica gel. The results suggested that lavender and rose solid by-products could be successfully utilized as bio sorbents for purification of textile industry waste waters.

Key words: *adsorption, by-products, lavender, rose, textile dyes.*

INTRODUCTION

The essential oil and, in particular, the rose and lavender essential oil industry, is one of the emblematic and widely represented among the agricultural industries in Bulgaria. Essential oil and medicinal raw materials from various families (*Rosaceae*, *Lamiaceae*, *Asteraceae*, etc.) are grown and processed. Bulgaria, together with Turkey, provide over 80% of the world's supply of rose oil, with the Bulgarian one having better indicators and, accordingly, the most sought after by the perfumery and cosmetics industry. Although there are over 200 species of roses, the following cultivated roses are mainly used to obtain aromatic products: *Rosa damascena* Mill., *Rosa gallica* L., *Rosa centifolia* L. and *Rosa alba* L. (Kovacheva et al., 2010). Due to the low content of essential oil in the raw material (for example, for *Rosa damascena* about 0.030-0.045%; for *Rosa centifolia* and *Rosa alba*, these amounts are even lower: 0.02% and 0.015-0.030%), after its extraction (through steam distillation, extraction with organic solvents or liquefied gases: freons, CO_2) large amounts of by-products are generated. In most cases, distilleries dump this biomass into the surrounding area, where it spontaneously ferments. However, these procedures disturb the ecological balance in the areas where these wastes are disposed of, both because of water pollution and because of the antibacterial action of biologically active substances (polyphenols, residual aromatic components) in the by-products. Moreover, this approach leads to the loss of valuable substances with potential applications in medicine, food, cosmetics, etc. industries. In order to find solutions for the utilization of waste raw materials from the essential oil industry, various approaches and methods have been developed in recent years - controlled composting or co-composting with biodegradable waste materials, extraction of valuable biologically active substances such as polyphenols, polysaccharides, etc., biosorption of various industrial pollutants - heavy metals, pigments, etc., gasification, feed additives, etc. (Schieber et al., 2005; Slavov et al., 2016; 2017). In most cases, these approaches can be integrated into combined methods that provide more complete utilization of waste raw materials (Slavov et al., 2017).

Textile industry, although worldwide developed, with incomes above trillion dollars, and more than 35 million employees, is among the manufactures which uses large amounts of water and causing serious environmental problems (Lellis et al., 2019). One of the main concerns for the environment is related to safely disposal of the wastewaters in the open water basins. Besides the substances used for bleaching, textile pretreatment, levelling and finishing agents, regulators of the pH, etc., the dyes are among the compounds causing mostly environmental pollution (Nicolai et al., 2021). Having usually phenolic character they are stable to light, temperature and biodegradation which makes them resistant and difficult to degrade. Furthermore, some of the products of degradation, also could pose a serious health risks for the humans and the environment (Crettaz et al., 2020). Globally, around 10,000 dyes are commercially used and their overall production is around 700,000 tons annually (Singh et al., 2017). Often, the particular companies have their own receipt and usually more than one dye is included in the formulations. Direct discharge is related to increased biochemical oxygen demand and chemical oxygen demand (COD), and often leads to heavy pollutions (Dehghani et al., 2018). Therefore, effective methods to remove the dyes from wastewaters are needed. The approaches used for purification of the wastewaters comprises of coagulation, flocculation, precipitation, membrane filtration, electrochemical techniques, bioremediation by microorganisms and conventional biological treatments (activated sludge) (Amalina et al., 2022; Lellis et al., 2019). However, these techniques in many cases lack efficiency or necessitate high initial investments and expensive operative costs (Kumar et al., 2011).

The removal of pollutants from industrial wastewater streams is a major problem for industry, agriculture and society. Increasing water pollution is suggested by studies on water treatment, with the primary importance being the removal of heavy metals and organic pollutants from industrial wastewater and increasing its potability (Abdel-Ghani & Elchaghaby, 2007; Karthik et al., 2014). The adverse effects of heavy metals, even in small amounts, are diverse and include destruction of the immune system, interference with the synthesis of some vital enzymes, cancer and nervous disorder, especially in children (Karthik et al., 2014). Natural materials that are available in large quantities or some wastes from agricultural activities can potentially be used as low-cost adsorbents, as they represent resources that are renewable, widely available, and environmentally friendly. A large number of studies have focused on the application of low-cost natural adsorbents, including carbonaceous materials, agricultural wastes and by-products, which are recognized as a potential alternative to conventional technologies such as sedimentation, ion exchange, solvent extraction and membrane technologies for the removal of heavy metals and organic pollutants from industrial wastewaters, as these processes have technical and/or economic limitations. The plant biomasses had been found effective in removing trace metals and organic pollutants from the environment (Abdel-Ghani & Elchaghaby, 2007).

Adsorption is a process of pollutant removal that is among the most promising and effective techniques for wastewaters purification (Wong et al., 2020). The adsorbents could be often regenerated and reused (Nascimento et al., 2014). Besides, additional benefit is utilization of by-products from the food and agricultural industry (essential oil industry for example) as natural, biodegradable, cheap and readily available adsorbents (Slavov et al., 2017).

Available data on the use of rose and lavender industrial by-products for adsorption of pollutants (heavy metals, such as copper, chromium, oil, zinc, textile organic materials, etc.) indicate that these materials can be used for wastewater treatment (Abdel-Ghani & Elchaghaby, 2007; Bhatti et al., 2011; Iqbal et al., 2013). Rabbani et al. (2016) investigated applicability of rose watering waste (the residues after industrial water-steam distillation for essential oil production). They found that the rose by-products could be successfully used as adsorbent and optimum conditions toward maximum removing value of COD and the color were contact time of 60 and 45 min for bulk and nano-biosorbent, respectively, as well as pH = 5.0 and biosorbent dosage of 2 g/L.

Therefore, the aim of the present study was focused on utilization of by-products from the essential oil industry (rose and lavender solid residues) as natural, renewable and biodegradable adsorbents for removal of industrial textile spent dyes from waste-streams. This approach also allows searching for alternative methods for valorization of the industrial by-products of the rose and lavender essential oil industry.

MATERIALS AND METHODS

Lavender (bio-certified *Lavandula angustifolia* Mill., Sevtopolis var.) - L and rose (bio-certified *Rosa damascena* Mill.) - RD, by-products from industrial steam distillation of fresh plant material were provided by the ECOMAAT distillery (Mirkovo, region of Sofia, Bulgaria; crop 2021).

The textile dyes (initial and spent ones) were provided (as a ready to be used solutions) by E.Miroglio EAD - Sliven, Bulgaria.

The rose and lavender by-products were collected from the still after the end of industrial treatment and dried. The dried solid residues were washed with distilled water (100 g with 4×400 mL deionized water). The residual solid mass was dried, milled and sieved. The same procedure was followed for RD and L by-products washing with acetone, 70% ethanol and 0.1 N hydrochloric acid. For experiments RD and L fractions with particle size ranging from 50 μm to100 μm were used.

The dyes' adsorption was performed as follow: 20 mL dye solution was added to 1 g of RD or L residue in a 50 mL centrifuge tube and the tubes were placed on a laboratory shaker MLW THYS 2 (VEB MLW Labortechnik Ilmenau, Germany). The shaker was started (100 rpm) and at a specified time (2.5, 5, 10, 20, 30, 40, 60, 90 and 120 min) a centrifuged tube was removed from the shaker and the content was filtered through a paper filter and further through a syringe filter CA 0.45 μm (Isolab, Germany). Adsorption of the filtrate was measured in a 1 cm cuvette at 489 nm for dye 2, 578 nm for dye 3, 600 nm for dye 4 and 546 nm for dye 6, employing LLG-uniSPEC 2 UV-Vis spectrophotometer (LLG Labware, Germany). The concentration of the remaining after the adsorption dyes was calculated using a calibration curves, prepared with solutions of the dyes with known initial concentrations and proper subsequent dilutions.

The analyses were run in triplicate, and the data were given as mean values. Statistical significance was detected by analysis of variance (ANOVA, Tukey's test; value of $p<0.05$ indicated statistical difference).

RESULTS AND DISCUSSIONS

Removal of four type of dyes were studied in the present investigation: two reactive type dyes, one dispersed for polyesters and one metal-complex. In order to check the applicability of RD and L by-products as adsorbents, the dye № 2 (dispersed for polyesters) was firstly used before dyeing process (solution ready for dyeing provided from the manufacturer) and comparison was made with the same dye but used in the dyeing process (spent dye). The characteristics of the investigated dye mixtures are presented in Table 1.

Table 1. Characteristics of the textile dyes, objects of the investigations

	Dye	C, mg/ml	pH	pH spent dye
2	Dispersed - polyester (Dianix Flavin XF; Dianix Scarlet XF; Dianix Marine XF)	3.21	4.14	4.25
3	Reactive - cotton (Levafix Gelb CA; Levafix Rot CA; Levafix Marine CA)	2.57	6.09	10.79
4	Reactive - wool (Kemazolan Giallo W-CE/01; Lanasol Red CE; Lamasol Navy CE)	4.31	4.63	4.77
6	Metal-complex (Kemsetl Giallo 2R; Kemaset Ross G; Kemaset Marine Blue R/02)	4.77	5.80	5.12

Influence of the contact time on the adsorption of textile dyes

One of the most important parameter considering the adsorption as a physical process is the time when the adsorbent and the pollutant interact between each other. For this reason first the contact time of the adsorbent with the dyes was investigated. The contact time determines the equilibrium of the dye adsorption and is important from both economic and ecological matters (Figure 1). In the beginning of the adsorption the process takes place quickly because of the great number of unoccupied sites at the surface of the L and RD by-products. Sharp increase of the adsorbed azo dye was observed approximately until the 5th min for all the samples, after that clearly equilibrium was established.

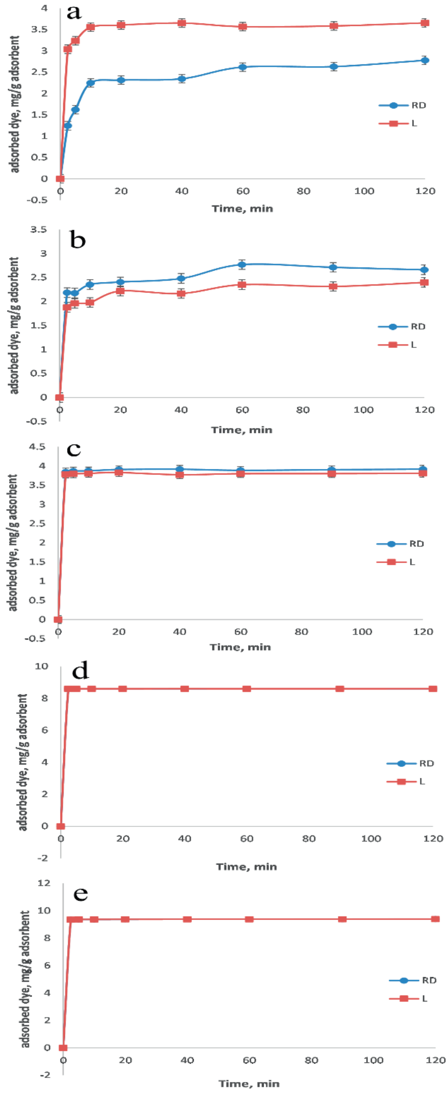

Figure 1. Influence of the contact time on the adsorption of textile dyes (amount of the adsorbent: 1 g; temperature: 20ºC; shaking at 100 rpm); a) initial dye 2; b) spent dye 2; c) spent dye 3; d) spent dye 4; e) spent dye 6

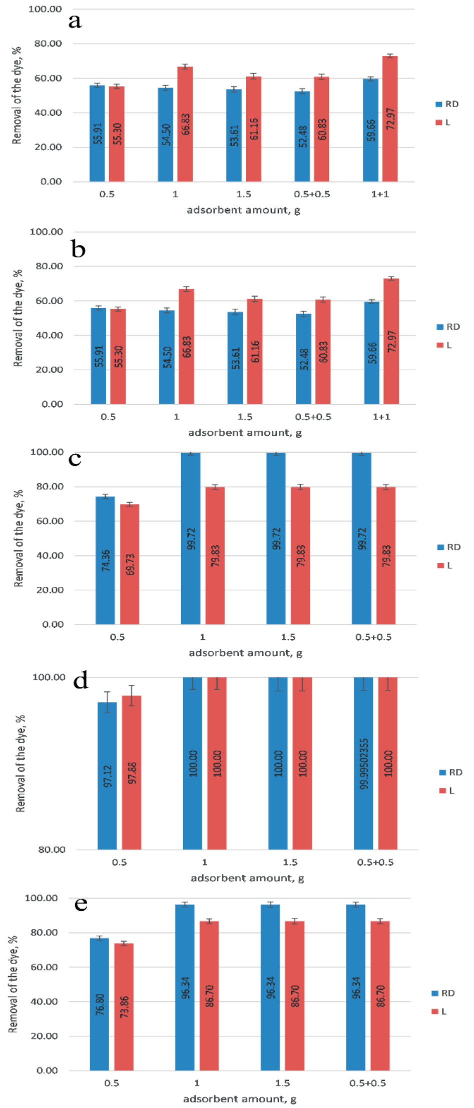

Figure 2. Influence of the amount of the adsorbent/re-adsorption on the adsorption of textile dyes (contact time 20 min; temperature: 20ºC; shaking at 100 rpm); a) initial dye 2; b) spent dye 2; c) spent dye 3; d) spent dye 4; e) spent dye 6

After 120 min when the process was stopped the efficiency of the adsorption was: for initial dye 2 - 58% for RD and 66% for L; for spent dye 2 - 60% for RD and 68% for L; for spent dye 3 - almost 100% for RD and 81% for L; for spent dye 4 - 100% for both adsorbents; for spent dye 6 - 94% for RD and 83% for L.

Similar results were obtained by Rabbani et al. (2016) investigating adsorption capacity of nanoparticles obtained from rose watering waste (*Rosa Damascena*). Clearly, the adsorption efficiency depended from the type of the dye mixture and for dye 2 L had the better results, while for dyes 3 and 6 - RD.

Influence of the amount of the adsorbent/re-adsorption on the adsorption of textile dyes

The amount of adsorbent plays important role and usually higher amounts lead to better effectiveness in the percentage removal of the pollutant. This, of course is related also to the higher operating costs and when possible lower amounts of the adsorbing material should be employed. Besides, the spent adsorbent have to be further treated in order to be safely disposed. The best results were achieved (Figure 2) using 1 or 1.5 g RD adsorbent (single adsorption; almost 99% removal for spent dye mixtures 3, 4 and 6 was achieved). The maximum possible single amount of adsorbent was 1.5 g - above this amount retention of the water phase inside the adsorbent did not allowed to obtain purified water solutions.

Consecutive adsorption was also tested and it was found that for both adsorbents doses of 0.5+0.5 g and 1+1 g adsorbents showed better results only for dye 2. One of the problems arising using consecutive adsorption was related to the higher water retention capacity of the R and L, having strong hydrophilic character. At doses 1.5+1.5 g adsorbents no water phase was able to obtain after adsorption. In our experiments single adsorption procedure with 1 g or 1.5 g of both adsorbents showed comparable results with consecutive adsorption, suggesting that one step was enough. For dye 2, however, the results were not the best ones, and additional steps or other adsorbents should be employed.

Influence of the temperature on the adsorption of textile dyes

The results for the effect of temperature on the effectiveness of adsorption for both adsorbents was ambiguous for different dye mixtures. For dye number 2 was even different for the spent and initial dye mixtures. For the initial dye the best removal was observed at 40°C. At 100°C the removal was around 80% but at this elevated temperatures it is possible to have degradation of some of the dyes which would gave misleading results. For the spent dye 2 the adsorption was negatively influenced by the temperature increase (Figure 3). For the dye 3 again a negative trend with temperature increase was observed. Dye mixtures 4 and 6 were not influenced by the temperature and this is the best possible situation, having in mind that operating at lower possible temperatures (ideally at ambient temperature) is beneficial for energy saving.

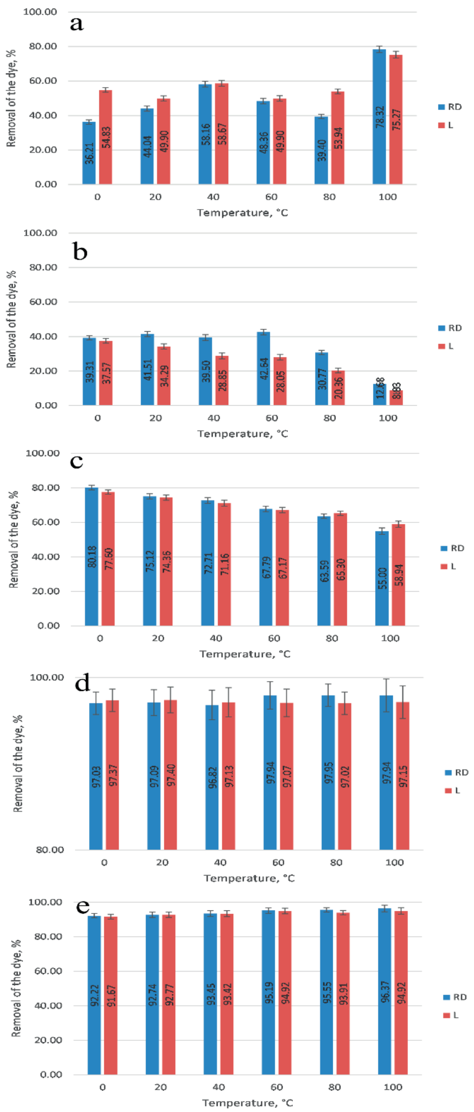

Figure 3. Influence of the temperature on the adsorption of the textile dyes (contact time 20 min; amount of the adsorbent: 1 g; shaking at 100 rpm)

Tosi Pelosi et al. (2013) observed that with temperature increase the adsorption of acid orange 7 dye by *Salvinia natans* biomass was favored. Balarak et al. (2020) had similar conclusions on the influence of the temperature on the adsorption of acid orange 7 by canola wastes. However, Wu et al. (2018) observed ambiguous effect of the temperature on the adsorption of acid orange 7 on waste brewery's yeast. The results obtained by the authors might be due to the increased rate of desorption with temperature increase. All these observations suggested that the temperature influences the adsorption process most of the time depending from the adsorbent, pollutant and the matrix. No significant effect (favoring or suppressing) for the influence of the temperature on some of the particular adsorbent (RD or L) used was observed in the present study.

Influence of the type of the adsorbent on the adsorption of the textile dyes mixtures

In the next experiments the effectiveness of the RD and L to remove dyes from wastewaters was compared with several known commercial adsorbents: activated carbon, Al_2O_3 and silica gel (Figure 4). Also comparison of the adsorption applicability with pretreated RD and L by-products (with 70% ethanol, acetone and 0.1 N hydrochloric acid) was made. The best results for adsorption effectiveness of the initial dye 2 showed the silica gel but for the adsorption of the mixture of spent dye 2 the acid washed lavender by-product (L_Res_AE) was the most effective followed by Al_2O_3. RD had better adsorption capacity than L but in general their adsorption effectiveness towards dye number 2 was limited. Spent dye 3 was well absorbed by most of the adsorbent used and again L_Res_AE was the most effective. For the spent dyes 4 and 6 activated carbon, silica gel and L_Res_AE showed the highest effectiveness. The results suggest that acid washing is generally beneficial to the applicability of the residues as adsorbents. These observations are in accordance with the results for the influence of the pH on the adsorption of Acid Orange 7 and Remazol Black 5 reactive dyes from aqueous solutions (Hamzeh et al., 2012). The effectiveness at lower pH is related to the net charge of the adsorbents. At lower pH medium the functional groups in the plant matrix will be protonated and attraction forces will favor retention of the dyes.

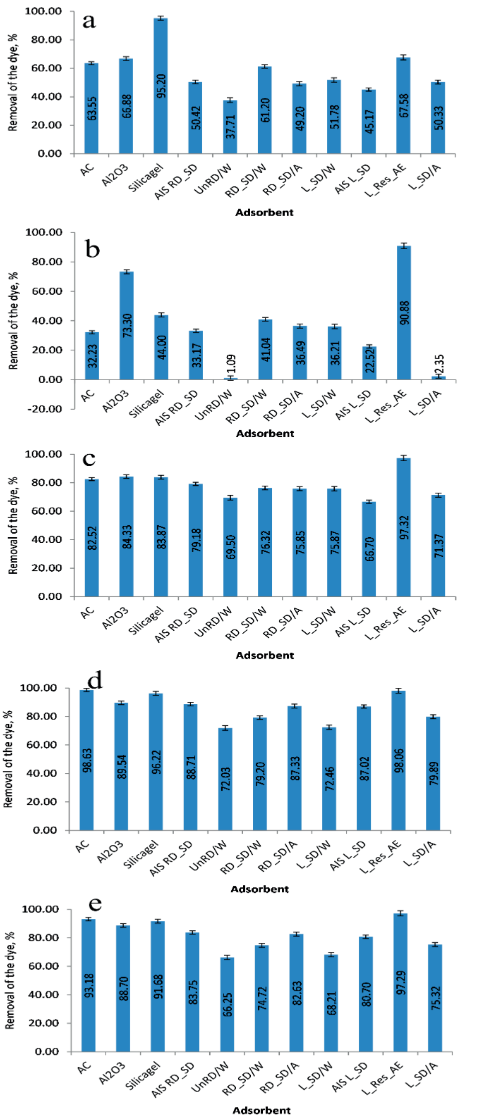

Figure 4. Influence of the type of the adsorbent on the adsorption of textile dyes (contact time 20 min; amount of the adsorbents: 1 g; shaking at 100 rpm). AC - active carbon; Al_2O_3; Silicagel; AIS RD_SD - rose by products washed with 70% ethanol; UnRD/W - dried rose petals (non-distilled) washed with distilled water; RD - steam-distilled rose by-products washed with distilled water; RD_SD/A - rose by products washed with acetone; L - steam-distilled lavender by-products washed with distilled water; AIS L_SD - lavender by-products washed with 70% ethanol; L_Res_AE - steam-distilled lavender by-products washed with 0.1 N HCl; L_SD/A - lavender by products washed with acetone

The results from the experiments suggested that RD and L by-products could be successfully applied as a bioadsorbent for removal of textile dyes from industrial water solutions. The residues generated after adsorption of the dyes could be used as a substrate for further higher fungi degradation and production of mycelium based biocomposites (Angelova et al., 2021). Due to the phenolic nature of the most of the dyes, the higher fungi, able to produce lignocellulosic enzyme complexes, could successfully degrade the organic pollutants and completely eliminate the hazardous threat for the nature these dyes pose.

CONCLUSIONS

The present study explored the application of two industrially generated by-products of the essential oil rose and lavender industry as bioadsorbents. The solid by-products are cheap, renewable, biodegradable and natural materials, which could be successfully applied for adsorption of organic pollutants, such as the spent dyes in the industrially generated textile wastewaters. The influence of contact time, amount of the adsorbent, temperature, and initial pretreatment of the adsorbents was investigated. Comparison of the effectiveness of the RD and L as adsorbents with activated carbon, Al_2O_3, and silica gel was made. It was found that equilibrium was established after 5-10 min contact time. The influence of the temperature on the dyes' removal was ambiguous and for this reason the ambient temperature was chosen for adsorption experiments (20°C). Previous studies of our team on the adsorbents particle size influence suggested that particles around or less than 50 μм showed the best results for adsorption effectiveness. Acid washing (with 0.1 N hydrochloric acid) as a pretreatment lead to the best results for removal of textile dyes from the medium. The resulted adsorbents had adsorption capacity similar to that of activated carbon and silica gel as a well-known commercially available adsorbents. The best adsorption effectiveness showed both acid-washed rose and lavender adsorbents, as lavender acid washed by-product adsorbed almost all of the dyes in spent formulations 3, 4 and 6.

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EFECTS OF SOME DRYING METHODS ON THE CONTENT IN BIOACTIVE COMPOUNDS IN SEA BUCKTHORN BY-PRODUCTS

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Abstract

Recent research has shown that the consumption of sea buckthorn (Hippophae rhamnoides) berries has many benefits for human health. Large amount of waste which result from sea buckthorn berries processing, yet containing significant quantities of bioactive substances, is being the subject of numerous latest research studies. Sea buckthorn by-products represent a valuable source of bioactive compounds that could be used for their favorable nutritional and functional properties in the pharmaceutical, cosmetic and food industries.
The aim of this work is to establish a method for drying process of sea buckthorn by-products in order to obtain a powder from dry sea buckthorn waste with minimum loss of the bioactive substances. For this purpose, sea buckthorn by-product (peel and seeds) was dried at different temperatures (30˚C, 40˚C, 50˚C and 60˚C) by conventional hot air drying versus lyophilization method and then the antioxidant activity (DPPH), the content of total polyphenols and ascorbic acid content were determined.

Key words: *bioactive compounds, by-products, conventional drying, lyophilization, sea buckthorn.*

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*) is a nutritionally valuable plant which is mostly consumed in the form of whole fruits. It contains a number of bioactive compounds such as polyphenols, flavonoids, organic acids, vitamin C, polysaccharides, unsaturated fatty acids and various natural amino-acids, thus it is used as a valuable food product but at the same time as medicine, outcomes which have been emphasized in the studies carried out until now (Krejkarova et al., 2015; Nilova and Malyutenkova, 2018; Sotler et al., 2019) The development of research and interest in this plant led to the extend cultivation of seabuckthorn from the spontaneous flora into crops. However, the expansion of sea buckthorn crops also influenced its processing. With easy adaptability and good profitability, the industrialization of sea buckthorn fruits presented great interest to the economic agents. Approximately 150 species, subspecies and varieties of sea buckthorn have been identified; they differ in shrub habitat, appearance of berries and their use-value (Ciesarova et al., 2020). Sea buckthorn berries are attractive due to their nutritional properties, being scientifically recognized for their content in vitamin C, carotenoids, flavonoids, sterols and tocopherols (Maheshwari et al., 2011; Olaru & Popa, 2019; Ciesarova et al., 2020). Sea buckthorn juice is the most consumed product obtained from sea buckthorn fruits processing. It has a long shelf life without needing any heat treatment, consequently there is no influence on its bioactive compounds content.

Effective valorisation of agri-food industrial wastes/by-products targets to contribute to an enhanced economy and also to minimize the negative impact on the environment, with positive effect on ensuring food security (Bhat, 2021; Kumar et al., 2021b).

Studying the nutritional aspects of sea buckthorn by-products could lead to new opportunities for obtaining nutraceuticals and natural functional food ingredients at low prices. The content of bioactive compounds in sea buckthorn by-products and their use in functional foods development have been studied in recent years. Most of the available literature has focused on evaluating the effect of the extraction protocol or method used for plant extracts preparation. However, the method for post-extraction conditioning is also important for assessing the content in bioactive compounds, especially since recent studies have reported that the drying method used to process the pre-extraction of plant biomass affects its bioactivity and chemical composition (Altemimi et al., 2017; Fernandes et al., 2018; Pham et al., 2015). Lyophilization is the "gold standard" for drying plant extracts, playing an important role in preserving their quality and extending their shelf life (ElNaker et al., 2021). Lyophilization limits oxidative modifications of metabolites because the oxygen concentration is deficient in vacuum (Papageorgiou et al., 2008). However, compared to other methods of drying plant extracts, lyophilization is more expensive due to equipment, materials and operational costs (Soquetta et al., 2018).

The alternative to modern freezedrying is conventional drying, by hot air, in grate or screen dryers, which is frequently used due to the low cost and availability of the equipment.

The main purpose of this work was to compare both drying methods for the sea buckthorn by-products and to establish which is the optimal drying method with optimal working parameters in order to maintain the content on chemical and nutritional substances. Due to its properties, sea buckthorn by-products could be used as a valuable functional ingredient in the new product development process.

MATERIALS AND METHODS

The plant material used in the experiments

In this study three organic sea buckthorn varieties, respectively Mara, Clara and Sorana were analyzed for water activity, dry matter, total polyphenol content, antioxidant activity and ascorbic acid content in order to investigate the effects of drying methods on the bioactive compounds content of the by-products obtained after sea buckthorn processing.

Pretreatments applied to sea buckthorn by-products

A series of preliminary test were performed in order to establish a juice squeezing method for sea buckthorn berries, as to prepare the sample for lyophilization by preliminary freezing and optimizing the way that samples could be better arranged into the lyophilization chamber.

In order to squeeze the sea buckthorn fruits, tests were carried out in parallel with two constructive types of fruit juicer: a centrifugal juicer and an auger juicer.

The centrifugal juicer has a series of knives arranged circularly in the centre of a sieve in the shape of a truncated cone. The fruits are first cut into very small pieces and thrown by centrifugal force into the sieve. This process happens very quickly and involves exposure of the juice droplets to air (oxygen) and metal (sieve). The fruit squeezing process, in this case, is a fast one.

The squeezing method for the auger juicer is by mastication and pressing. The fruits are cut by the auger at the top of the spindle, then crushed in the middle of the auger spindle, and the actual pressing is done at the bottom. The fruit squeezing process in this case is a slow one.

The by-products resulted after the sea buckthorn processing was conditioned by lyophilization respectively conventional drying, at different temperatures 30^0C, 40^0C, 50^0C, 60^0C.

Setting up the conventional drying and freeze-drying methods

The conventional drying was carried out with a Biovita Deluxe-10 food dehydrator (Figure 1). Regarding the setting up of sea buckthorn by-products conventional drying, the working parameters were tested at different temperatures for certain time intervals as follows: 60°C for 9 hours, 50°C for 12 hours, 40°C for 15 hours, respectively 30°C for 19 hours.

The sea buckthorn by-products lyophilization was carried out with a Labconco - FreeZone lyophilizer (Figure 1), which has a capacity of 2.5 litres. It allows lyophilization of moderate volume samples at a temperature of -50°C, which is why, following optimization tests of the freezing process as a preliminary stage in the lyophilization process, it was established to store the samples at -35°C for 24 hours. It is preferable that the freezing temperature of the samples is as close as possible to the operating temperature of the collector of the lyophilizer.

The collector is made of stainless steel and can hold approximately 2.5 liters of ice before defrosting.

Food dryer	Labconco Freeze dryer

Figure 1. The equipment used for air drying/liophilization of sea buckthorn by-products

Refrigerator Hotpoint	Climate chamber Feutron KPK 200
Freezing temperatures tested: -18^{0}C, -20^{0}C, -24^{0}C	Freezing temperatures tested: -30^{0}C, -35^{0}C

Figure 2. Equipment used for freezing sea buckthorn by-product samples that were subjected to freeze drying

In order to establish the lyophilization technology of the sea buckthorn by-products, a series of parameters were tested, such as:
- collector temperature;
- the temperature of the shelf inside the lyophilization chamber;
- pressure, in order to obtain vacuum;
- the freeze-drying time.

The preliminary tests to establish the lyophilization parameters of sea buckthorn by-products were carried out on Mara variety.

- the temperature of the collector varied in the range -46°C-48°C;
- the temperature of the shelves in the freeze-drying chamber was kept constant at 60°C;
- the pressure varied between 0.200-0.800 mbar;

The lyophilization time tested was 24 hours, 48 hours, and 72 hours, respectively.
The by-products obtained were frozen, this being a mandatory preliminary stage in the lyophilization process. The low temperature allows the final product to be of higher quality. To optimize the freezing process, different freezing temperatures were tested. In the preliminary stage, the freezing of sea buckthorn by-products was tested in a Hotpoint refrigerator at freezing temperatures of -18°C, -20°C, and -24°C, respectively. Subsequently, the freezing of sea buckthorn by-products was tested in the Feutron climate chamber (Figure 2) which allowed freezing at -35°C. The freezing time of sea buckthorn by-products was also tested.

Each shelf of the lyophilization chamber can have its own temperature probe for the most accurate temperature monitoring. The shelves of the lyophilization chamber can be removed from the chamber to be pre-frozen and also have the pre-freeze function with a cooling system of about 240 watts. In order to optimize the operation of the lyophilizer, but also to avoid losing the freezing temperature of the samples, it was decided to start the lyophilizer before introducing the samples into the lyophilization chamber, so that the collector reaches the operating temperature before placing the samples on the shelves of the lyophilization chamber.
Regarding the arrangement of the samples on the lyophilizer shelves, the distribution of 4 glass Petri dishes (with a diameter of 90 mm) on each of the 3 shelves of the lyophilization chamber was preliminarily tested. Thus, the sea buckthorn by-products were previously distributed in the glass Petri plates, which were subsequently subjected to freezing at -35°C for 24 hours. In this way, temperature losses could be minimized when handling the samples, which involves moving them from the climate chamber to the lyophilization chamber.
In order to make more efficient use of the space in the lyophilization chamber, we resorted to replacing the 90 mm diameter Petri dishes that did not fully cover the surface of the shelves with aluminum trays that have a diameter of 25 cm. These aluminum trays have a number of advantages, including:
- allow a quick heat transfer;

- ensures uniform distribution of the sample, in a thicker layer, thus allowing lyophilization of a larger amount of sample;
- ensures efficient use of the space on the shelves of the lyophilization chamber;
- reduce the risk of scattering the sample inside the lyophilization chamber, during handling or depressurization;
- ease of handling samples;
- simpler and more effective sanitation;
- multiple use.

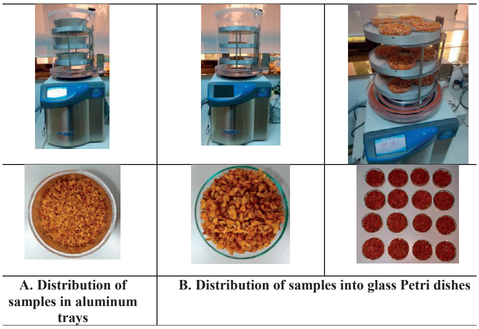

Figure 3. The arrangement of the samples in the lyophilizer

In order to highlight the effects of the freeze-drying treatment versus the conventional drying treatment on sea buckthorn by-products, the following indicators were analysed: vitamin C content, antioxidant activity and polyphenol content.

Antioxidant activity

The effect of antioxidant activity on 1,1-dipheny l-2- picrylhydrazyl (DPPH) was estimated according to the procedure described by Villaño et al. (2007), with some modification presented further. Briefly, 10 g of sample was macerated in 50 ml ethanol (75%) for 48 h in the dark, at room temperature. For each measurement, 0.05 ml filtered extract solution was added to 1.95 ml DPPH ethanolic solution and thoroughly homogenized, and incubated in dark at room temperature for 30 min. Sample absorbance was measured at 515 nm. Results were expressed as quercetin equivalents (QE) per 100g D.W.

Ascorbic acid content

The content of ascorbic acid was determined by extracting 10 g of sample in 100 ml of 2% oxalic acid. The extract was filtered and 2 ml from the extract solution, 1 ml oxalic acid 2%, 5 ml buffer solution, 2 ml indophenol (2, 6-Dichlorophenol Indophenol) and 20 ml xylene, were placed in a centrifuge tube and centrifuged for 20 min at 5°C and 9000 rpm. The absorbance of the samples was measured 500 nm and the results were expressed as mg ascorbic acid/100 g D.W.

Total polyphenolic content

Total content of polyphenols (TP) was determined using the Folin-Ciocalteu method. Briefly, for each measurement, 1.58 ml distilled water, 20 µl filtered extract solution (10 g of fruit macerated in 50 ml ethanol (75%) for 48 h in the dark at room temperature), and 100 µl Folin-Ciocalteu reagent were mixed and then 300 µl Na_2CO_3 (20%) was added. The solutions were mixed and stored in the dark at room temperature for 2 hours. Sample absorbance was measured at 765 nm. Total polyphenol concentration was expressed as mg/L Gallic acid equivalents (GAE) per 100 g D.W.

Statistical analysis

All determination was performed in duplicate. The obtained data was statistically analysed using Microsoft Excel 2017. In all tests, it was considered the significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

In order to highlight the effects of the freeze-drying treatment versus the conventional drying treatment on sea buckthorn by-products, the following indicators were analysed: vitamin C content, antioxidant activity and polyphenol content. Following the preliminary tests made with Mara sea buckthorn variety (Table 1), freeze-drying operating parameters were established as follows:
- vacuum: 0.270 mbar;
- collector temperature: - 48°C;
- freeze-drying time: 24 hours;
- temperature of the shelf in the freeze-drying room: 60°C, 50°C, 40°C, respectively 30°C.

Afterwards by-products from all three sea buckthorn varieties tested (Mara, Clara, Sorana) were freeze-dried and the notations used are presented in the Table 2.

Table 1. Preliminary tests in order to establish the lyophilization parameters of sea buckthorn by-products

No. crt.	Sample	Pretreatments	Liophylization parameters				
			Collector temperature (^{0}C)	Vacuum (mbar)	Shelf temperature (^{0}C)	Freeze drying time (hours)	Final moisture content (%)
1.	MARA variety	Fruits squeezing, By-products preliminary freezing at -35^{0}C for 24 hours Ui = 53,285 %	- 46.7	0.794	60	24	8.755
2.			- 47	0.233	60	48	7.439
3.			- 47.6	0.237	60	24	10.963
4.			- 47.6	0.240	60	24	9.082
5.			- 46.9	0.297	60	48	8.491
6.			- 47.3	0.290	60	72	8.310

Table 2. The notation used in the presentation of results

No. crt.	Samples analyzed	Coding of the samples
1.	Sea buckthorn by-product from the Mara variety lyophilized at 30^{0}C temperature on the shelf of the lyophilization chamber	LM 30
2.	Sea buckthorn by-product from the Mara variety lyophilized at 40^{0}C temperature on the shelf of the lyophilization chamber	LM 40
3.	Sea buckthorn by-product from the Mara variety lyophilized at 50^{0}C temperature on the shelf of the lyophilization chamber	LM 50
4.	Sea buckthorn by-product from the Mara variety lyophilized at 60^{0}C temperature on the shelf of the lyophilization chamber	LM 60
5.	Sea buckthorn by-product from the Clara variety lyophilized at 30^{0}C temperature on the shelf of the lyophilization chamber	LC 30
6.	Sea buckthorn by-product from the Clara variety lyophilized at 40^{0}C temperature on the shelf of the lyophilization chamber	LC 40
7.	Sea buckthorn by-product from the Clara variety lyophilized at 50^{0}C temperature on the shelf of the lyophilization chamber	LC 50
8.	Sea buckthorn by-product from the Clara variety lyophilized at 60^{0}C temperature on the shelf of the lyophilization chamber	LC 60
9.	Sea buckthorn by-product from the Sorana variety lyophilized at 30^{0}C temperature on the shelf of the lyophilization chamber	LS 30
10.	Sea buckthorn by-product from the Sorana variety lyophilized at 40^{0}C temperature on the shelf of the lyophilization chamber	LS 40
11.	Sea buckthorn by-product from the Sorana variety lyophilized at 50^{0}C temperature on the shelf of the lyophilization chamber	LS 50
12.	Sea buckthorn by-product from the Sorana variety lyophilized at 60^{0}C temperature on the shelf of the lyophilization chamber	LS 60
13.	Sea buckthorn by-product from the Mara variety conventionally dried at 30^{0}C	CM 30
14.	Sea buckthorn by-product from the Mara variety conventionally dried at 40^{0}C	CM 40
15.	Sea buckthorn by-product from the Mara variety conventionally dried at 50^{0}C	CM 50
16.	Sea buckthorn by-product from the Mara variety conventionally dried at 60^{0}C	CM 60
17.	Sea buckthorn by-product from the Clara variety conventionally dried at 30^{0}C	CC 30
18.	Sea buckthorn by-product from the Clara variety conventionally dried at 40^{0}C	CC 40
19.	Sea buckthorn by-product from the Clara variety conventionally dried at 50^{0}C	CC 50
20.	Sea buckthorn by-product from the Clara variety conventionally dried at 60^{0}C	CC 60
21.	Sea buckthorn by-product from the Sorana variety conventionally dried at 30^{0}C	CS 30
22.	Sea buckthorn by-product from the Sorana variety conventionally dried at 40^{0}C	CS 40
23.	Sea buckthorn by-product from the Sorana variety conventionally dried at 50^{0}C	CS 50
24.	Sea buckthorn by-product from the Sorana variety conventionally dried at 60^{0}C	CS 60

Regarding the setting up of conventional drying parameters for sea buckthorn by-products, the drying options were tested at different temperatures for certain time intervals that are specified in the Table 3 for all three varieties of studied sea buckthorn (Mara, Clara and Sorana). In terms of the completion of the drying process, the final moisture content of samples was considered the most important indicator, the results obtained ranging between 7.121% and 9.003%.

Table 3. Conventional drying treatments applied to sea buckthorn by-products

No. crt.	Sea buckthorn variety	Drying temperature (^{0}C)	Drying time (hours)	Initial moisture content of samples (%)	Final moisture content of samples (%)	Dry matter of samples (%)
1.	Mara	60	9	48.837	8.252	91.748
2.	Clara			50.276	7.712	92.288
3.	Sorana			51.551	8.006	91.994
4.	Mara	50	12	48.837	9.003	90.997
5.	Clara			50.276	8.155	91.845
6.	Sorana			51.551	8.352	91.648
7.	Mara	40	15	48.837	8.908	91.092
8.	Clara			50.276	7.149	92.851
9.	Sorana			51.551	7.899	92.101
10.	Mara	30	19	48.837	7.816	92.184
11.	Clara			50.276	7.548	92.452
12.	Sorana			51.551	7.121	92.879

Regarding the setting up of the sea buckthorn by-products lyophilization, freeze-drying options were tested at different temperatures for certain time intervals that are specified in Table 4 for all three varieties of sea buckthorn studied (Mara, Clara and Sorana). In terms of the completion of the freeze-drying process, the final moisture content of samples was considered the most important indicator, the results obtained ranging between 3.686% and 6.988%, lower values being recorded than in the case of conventional drying.

Table 4. Freeze-drying treatments applied to sea buckthorn by-products

No.	Sea buckthorn variety	Freeze-drying shelf temperature (^{0}C)	Freeze-drying time (hours)	Initial moisture content of samples (%)	Final moisture content of samples (%)	Dry matter of samples (%)
1.	Mara	60	24	48.837	4.587	95.413
2.	Clara			50.276	3.686	96.314
3.	Sorana			51.551	4.286	95.714
4.	Mara	50	24	48.837	5.711	94.289
5.	Clara			50.276	5.635	94.365
6.	Sorana			51.551	4.616	95.384
7.	Mara	40	24	48.837	6.063	93.937
8.	Clara			50.276	5.052	94.948
9.	Sorana			51.551	4.651	95.349
10.	Mara	30	24	48.837	6.988	93.012
11.	Clara			50.276	5.102	94.898
12.	Sorana			51.551	5.014	94.986

With respect to the appearance of the by-product obtained after squeezing, it was found that the one obtained with the auger juicer is devoid of juice or pulp, being entirely composed of very well pressed and crushed sea buckthorn skins and seeds (Figure 4B). As it can be seen in Figure 4A, which shows the appearance of the sea buckthorn by-products samples obtained after squeezing with a centrifugal juicer, it is insufficiently pressed, presenting pulp and juice remaining in the fruit.

A. obtained by squeezing with a centrifugal juicer (U = 53,285 %) B. obtained by squeezing with the auger juicer (U = 43,063 %)

Figure 4. The appearance of sea buckthorn by-products samples from the Mara variety

Regarding the antioxidant activity of the studied samples (Figure 5 and Figure 6), higher values were obtained for the conventionally dried samples compared to the lyophilized samples. In the case of freeze-dried samples, the highest values were obtained for the following temperature regimes: 40°C for the Mara and Sorana varieties and 50°C for the Clara variety. For conventionally dried samples, the highest antioxidant activity values were obtained for samples dried at 50°C for the Mara and Sorana varieties and 30°C for the Clara variety.

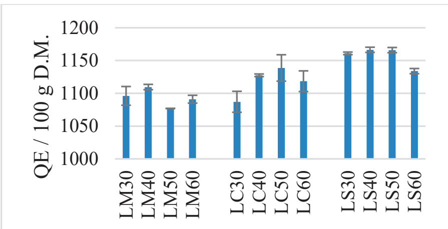

Figure 5. Antioxidant activity of freeze-dried sea buckthorn by-products

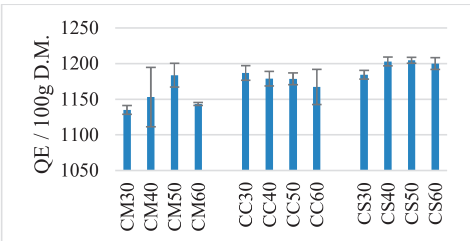

Figure 6. Antioxidant activity of conventionally dried sea buckthorn by-products

According to the results obtained from determination of the ascorbic acid content for the dehydrated sea buckthorn by-products, it was observed that the values obtained following the application of the studied drying regimes did not vary significantly, the ascorbic acid content showing close values.

Related to the working regimes studied, the highest content of ascorbic acid was obtained following the application of the temperature of 40°C in the lyophilization process, respectively conventional drying for the Mara variety, 50°C for the Clara variety and 60°C for the Sorana variety (Figure 7 and Figure 8).

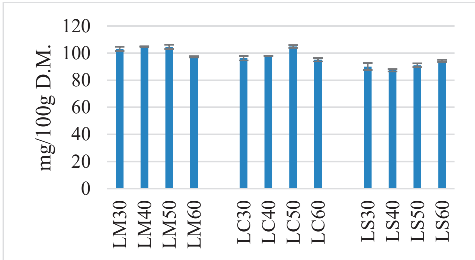

Figure 7. Ascorbic acid content of freeze-dried sea buckthorn by-products

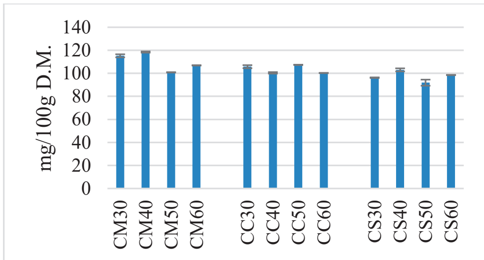

Figure 8. Ascorbic acid content of conventionally dried sea buckthorn by-products

Following the analysis of the results obtained in the case of the total polyphenol content determination (Figure 9 and Figure 10), no significant variations were observed between the two drying regimes, the values obtained for this parameter being similar.

In the case of freeze-dried samples, the highest values were obtained for the samples to which the following temperature regimes were applied: 30°C for the Mara variety, 40°C for the Clara variety and 60°C for the Sorana variety. Regarding conventionally dried samples, the highest polyphenol content was obtained for samples dried at 50°C for the Mara variety and 40°C for the Clara and Sorana varieties.

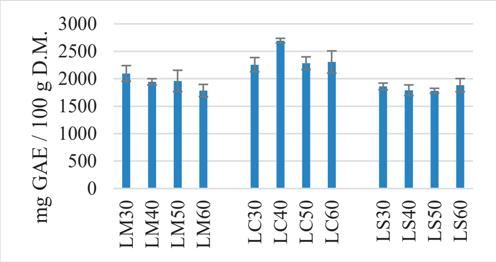

Figure 9. Polyphenol content of freeze-dried sea buckthorn by-products

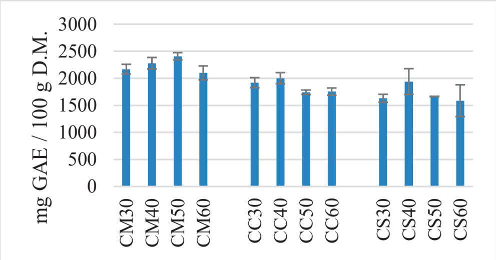

Figure 10. Polyphenol content for conventionally dried sea buckthorn by-products

CONCLUSIONS

Concerns related to the investigation of the most suitable conditioning method for sea buckthorn by-products continue to be a current topic, in order to keep the bioactive ingredient content so the important substances will not be degraded, for their further use in the food industry as potential functional ingredients. The effects of freeze-drying treatment were highlighted by comparison with conventional drying treatment.

Taking into account the results obtained, the best lyophilization regime, respectively drying, can be considered the regime with a working temperature of 40°C for 24 hours, generally obtaining high values in terms of the total polyphenols content, ascorbic acid content and the antioxidant activity for the samples subjected to this working regime compared to the other regimes tested for all the studied samples. Also, following preliminary tests, the following operating parameters were established for samples lyophilization:

- vacuum: 0.270 mbar;
- collector temperature: - 48°C;
- freeze-drying time: 24 hours.

From the point of view of the efficiency of sea buckthorn fruits juicing, it was found that the losses are lower when using the centrifugal juicer compared to the auger juicer, but in terms of the moisture and the appearance of the by-products, is recommended the use of the auger juicer.

Freezing at -35°C, in the climate chamber, is recommended as the preliminary stage of the lyophilization treatment, for the dehydration of sea buckthorn by-products.

After determining the antioxidant activity for the studied samples, higher values are observed for the conventionally dried samples compared to the lyophilized samples, for all varieties. The Sorana variety presented the highest values: CS30 - 1184 QE/100 g D.M., CS40 - 1203 QE/100 g D.M., CS50 - 1204 QE/100 g D.M., CS60 - 1200 QE/100 g D.M., the lowest values being recorded for the Mara variety, the samples subjected to the lyophilization operation: LM30 - 1096 QE/100 g D.M., LM40 - 1110 QE/100 g D.M., LM50 - 1077 QE/100 g D.M., LM 60 - 1091 QE/ 100 g D.M.

The results obtained from the determination of the ascorbic acid content were close, they are framed around the values of 100 mg/100g D.M., for the lyophilized and conventionally dried samples. A higher content of ascorbic acid is presented by the Mara variety, which conventionally dried at 30°C and 40°C shows values of 115.2 mg/100 g D.M., respectively 118.5 mg/100 g D.M.

The total content of polyphenols was influenced both by the variety of the samples and the process through which they were subjected. For lyophilization, the total content of polyphenols was assessed for the Clara variety samples, recording the highest values for LC30 - 2255 mg GAE/100 g D.M., LC40 - 2693 mg GAE/100 g D.M., LC50 - 2284 mg GAE/100 g D.M., LC60 - 2305 mg GAE/100 g D.M. Samples from the Clara variety subjected to conventional drying recorded the highest values: CM30 - 2169 mg GAE/100 g D.M., CM40 - 2278 mg GAE/100 g D.M., CM50 - 2407 mg GAE/100 g D.M., CM60 - 2100 mg GAE/100 g D.M. It is observed in both processes, as the temperature increases, the values decrease, the optimal temperatures being 30°C, 40°C, 50°C.

Laboratory research has shown that the application of the lyophilization process is suitable to preserve the bioactive properties of sea buckthorn by-products.

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TESTED SEED PRIMING METHODS TO STIMULATE THE GERMINATION OF *CITRUS LIMON* L.

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Abstract

The germination of exotic plants usually encounters difficulties. The research aim was to assess the effect of different seed priming methods on lemon germination. A number of 42 test tubes were filled with 11 cm of cotton wool and 1 cm of sterilized quartz sand. Each test tube contained one lemon seed. Three seed priming methods were tested respectively hardening with hot water, osmo-hardening with polyethylene glycol and hot water, hydro-priming, and a control treatment without priming methods. The assessments were made according to the BBCH scale of growth and development. The seeds from osmo-hardening treatments needed 42 days to reach stage 10 on BBCH scale, and the control needed two extra weeks. The hardening hot treatment and hydropriming seeds need 56 days to reach BBCH 09 secondary stage. A percent of 100% germination capacity was obtained to seeds from osmo-hardening, hydropriming and control treatments after 56 days. The most successful treatment in terms of secondary stages distribution was represented by the osmo-hardening, followed by hardening, then control, and the lowest result was in the hydro-priming treatment.

Key words*: breaking dormancy, hardening, hydro-priming, osmo-hardening; seeds.*

INTRODUCTION

Seed priming methods stimulate some metabolic processes necessary for germination without germination itself occurring (Marthandan et al., 2020). The most important processes that help rapid germination and increase seed tolerance to adverse environmental conditions are due to these seed priming methods most of the time (Raj and Raj, 2019; Singh et al., 2020). Seed priming was defined as pre-sown treatment in pure water or osmotic solutions, this hydration being sufficient to allow previous germinative metabolic processes but insufficient to allow radicle emergence through the seed coat (Bojović et al., 2022; Stoian et al., 2022).

The success of seed priming is influenced by the complex interplay of factors between plant species, the water potential of the priming agent, duration of pre-treatment, temperature, vigour, seed dehydration, and storage conditions of pre-treated seeds (Arun et al., 2022; Mal et al., 2019).

In general, the seed germination process in citrus is slow (Lugassi et al., 2020) and irregular due to the presence of inhibitory factors and the physical strength of the seed coat (Javed et al., 2022). One of the best seed priming method is represented by a controlled hydration pre-treatment in which the seeds are left to soak.

This process has an important role in improving the germination rate, germination uniformity, sometimes increasing the total germination percentage and more vigorous seedling growth (Thakur et al., 2022; Chakraborty and Dwivedi, 2021).

That is why certain seed pre-treatment techniques must be tested and selected to speed up or even halve the seed germination process.

Citrus fruits are currently consumed in large quantities due to the high content of ascorbic acid with therapeutic properties (Costanzo et al., 2020; Santos et al., 2021).

The most well-known and used citrus species is *Citrus limon* L. from the *Citrus* genus, *Rutaceae* family. Lemon is used for fruit consumption, due to the aromatic oils generally extracted from the mesocarp and seeds, as well as for ornamental purposes (Khan et al., 2021; Klimek-Szczykutowicz et al., 2020).

The aim of the study was to test three seed priming methods on lemon seeds to highlight the best method with high efficiency in germination time BBCH secondary stages.

MATERIALS AND METHODS

The experiment was set up at room temperature 20±2°C to simulate representative low-cost seed priming testing methods in order to obtain healthy and resistant seedlings in the shortest time. For this purpose, 78 seeds were used for seed priming techniques, respectively hardening with hot water (HH), osmo-hardening (O) with polyethylene glycol and hot water, hydro-priming (HY), and a control treatment (C) without priming methods. For the osmo-hardening priming technique, 2 ml of Fairy (polyethylene glycol) was added to 98 ml of warm water with a temperature of 50°C, then 12 seeds were placed in equal parts in the two Petri dishes. The osmotic solution was added to the Petri dishes until the seeds were completely immersed in the priming solution.

All seeds were left to soak for 24 hours

In the end, a number of 42 test tubes were filled with 11 cm of cotton wool and 1 cm of sterilized quartz sand. In order to highlight the tested seed priming methods, the germination capacity was assessed every two weeks at three time periods A1, A2, and A3. The first assessment was done after four weeks when the seeds start to germinate (A1). A2 - was the assessment at 6 weeks from the beginning of the experiment, and A3, respectively, at 8 weeks after the experiment starts.

The formula for germination capacity (%) was calculated following Bazai and Achakzai (2006):

*Germination capacity (%) = (Number of seeds germinated/total number of tested seeds) * 100.*

The detailed evaluation comprises also the secondary stages from the BBCH scale for lemon, separately for each treatment and all three assessments (Meyer, 2018).

RESULTS AND DISCUSSIONS

The lemon seeds' germination capacity was the highest at the third assessment A3 in the treatments with osmo-hardening, hydropriming, and control (Figure 1). After 4 weeks, at the first assessment (A1), the seeds from the osmo-hardening treatment presented a germination rate with 8% lower compared to the control. Then, this percentage increased after 6 weeks (A2) at 25% compared to the control. At the last assessment (A3) all the seeds from the osmo-hardening treatment succeed to complete the germination stage, at the same value recorded in control. The hardening with hot water was not so beneficial for lemon seeds. At the first assessment (A1), the difference compared to the control was by 50%. At the following evaluations the seeds started to germinate and the difference was 8% lower compared with the control treatment. The seeds from the hydropriming treatment were with 25% lower than the control in A1. After 6 weeks, the difference was with 8% (A2) and in the subsequent evaluation (A3), all the seeds succeeded to germinate (Figure 1).

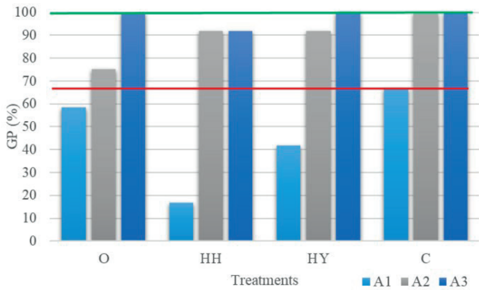

Figure 1. Germination capacity at all three assessments A1 after 4 weeks, A2 after 6 weeks, and A3 after 8 weeks for all treatments O-osmo-hardening, HH-hardening with hot water, HY-hydropring and C-control

All the seed priming methods gave positive results in terms of seed germination, except hydropriming (Figures 2, 3). At the first assessment, the osmo-hardening stimulated 41.67% of the seed to reach BBCH 03 stage. This number was lower with 25% compared with the control treatment, however, the control treatment seeds were not in other BBCH development stages. In the O treatment, 8.33% of the seeds evolved into both BBCH 05 and 06. Compared with the control, the O treatment produced seeds in 2 more developmental stages. At the second assessment, the same treatment (O), had 50% of the seeds in BBCH 03 and 8.33% in BBCH 05, BBCH 10, and BBCH 11.

The BBCH 03 was higher with 16.67 compared to control, while the BBCH 05 was lower with 25% compared with the control. Also, even the number of secondary stages present in the seed development of O and C was the same, the O jumped two secondary stages, so therefore the seeds were more evolved (Prasad et al., 2022). At the last assessment, 33.33% of the seeds were in BBCH 03 for O treatment. This percent was doubled for the same secondary stage from the control treatment. The secondary stages BBCH 05, BBCH 06 and BBCH 19 were each represented with 16.67% from the total share of the seeds. In the control treatment only BBCH 19 was present with 33.33% higher percent compared to O treatment. Only 8.33% of the seeds from O treatment were in BBCH 09 and the same percentage in BBCH 11. Hereby, 6 secondary stages were present, double the number present in the control treatment. This could be explained by the continuous beneficial effect of osmotic stress upon the seed and the stimulation in time of the embryo (Bafoil et al., 2019; Golmohammadzadeh et al., 2020).

The increased water temperature used for seed priming with (HH-hardening with hot water) did not had the same effect like the combination of osmotic solution with hot water (Figures 2, 3). The seeds at the first assessment (A1) of the HH treatment comprised 8.33% of the seeds in BBCH 03 and the same share reached BBCH 06. Only these two secondary stages were therefore present, with one extra compared with the control treatment. The second assessment (A2) highlighted 41.67% seeds in BBCH 05 with 8.34% higher than in control. The secondary stage BBCH 06 was represented by 33.33% of the seeds with 16.67% higher than the control. Also, the seeds in BBCH 03 were represented in a share of 16.67%, this time lower with 16.67% compared to control. It could be observed that even if the total germination capacity was higher at the second assessment in the control treatment, the HH seed priming method gave a fast development of the embryo (Raj and Raj, 2019; Dinesha et al., 2022; Arun et al., 2022). At the end of the experiment, the seeds from HH treatment were in BBCH 05 and BBCH 19 (33.33%), in BBCH 06 (16.67%) and in BBCH 09 (8.33%). Compared with the control, only BBCH 19 was with 16.67% lower in HH. The other two secondary stages present in the control treatment were BBCH 03 (66.67%) and BBCH 10 (16.67%). Therefore, the majority of the seeds could be considered over-evolved compared with the control treatment.

The hydropriming treatment (HY) had the closest results with the control treatment, being less efficient for lemon seeds (Figures 2, 3). At the first assessment (A1), the seeds were in BBCH 03 in a share of 41.67%, with 25% lower compared to control. At the second assessment (A2) in HY treatment the seeds were in 3 secondary stages BBCH 03 (41.67%), BBCH 05 (16.67%) and BBCH 06 (33.33%), missing BBCH 09, compared to control. These seed percentages share in HY were with 8.34% higher compared to control for BBCH 03, with 16.66% lower for BBCH 05 and with 16.66 higher for BBCH 06. At the last assessment (A3), the treatments shift trends compared with the previous one. From the 4 secondary stages present in HY treatments, a share of 33.33% of the seeds were in BBCH 03 and BBCH 19. The differences from the control were twice lower for BBCH 03 and with 16.67% lower for BBCH 19. Then, 25% of the seed from HY were in BBCH 05, and for the control treatment only 16.67% were in BBCH 10.

All tested treatments reached BBCH 19 after 8 weeks from the experiment start (Figures 2, 3). This aspect sustains the potential development of lemon seeds to succeed the same BBCH stage, independent to treatment. The differences between treatments are visible in the share of seeds that reach this final development stage, with more than 50% in control treatment. Both HH and HY treatments show a similar trend in BBCH stages at the end of the experiment, with BBCH 09 and 19 present. Compared to control, these two treatments present a shift only in BBCH 09 to BBCH 10 (control).

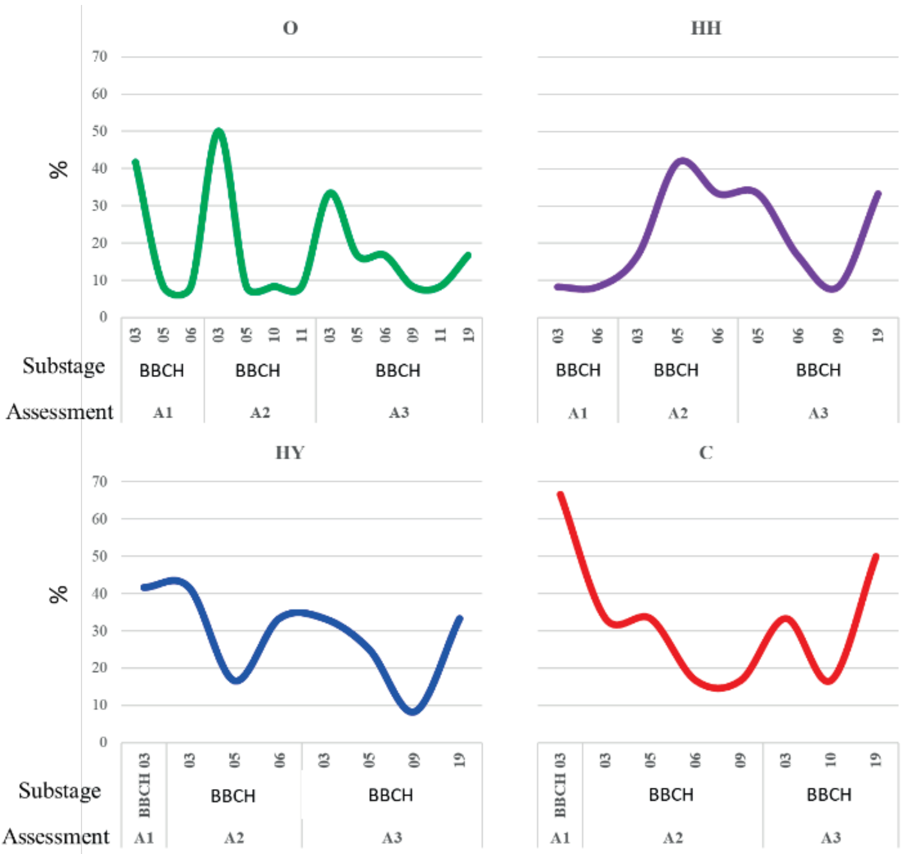

Figure 2. The percentages of germinated seeds for each BBCH sub-stages at all treatments from all three assessments

Figure 3. Phenological growth stages and BBCH assessed stages for *Citrus* spp. L. BBCH 03 - end of bud swelling: green scales slightly separated; BBCH 05 – radicle emerged from seed; BBCH 06 - elongation of the radicle; BBCH 09 - green leaf tips visible; BBCH 10 - first leaves separating: green scales slightly open leaves emerging; BBCH 11 - first leaves visible; BBCH 19 - first leaves fully expanded; classification used after Meier (2018)

The presence of BBCH 05, 06, 09, and 19 in HH indicates a gradual development evolution stimulated by this treatment, while the presence of 03 in HY-treated seeds indicates a slower development induced by this treatment (Figures 2, 3). An interesting case is visible in the O treatment, with BBCH 03, 05, 06, 09, 11, and 19 present at the end of the experiment. This indicates a step-by-step evolution of the germination process, with seeds gradually reaching each BBCH stage. The presence of numerous BBCH stages in this treatment sustains it as a solution to forecast the germination process, with the gradual development of embryos and plants.

CONCLUSIONS

The germination capacity was the highest in the control treatment starting with the day 42 of the experiment. This assessed parameter classify the priming methods on the second place the osmo-hardening, then hydropriming and at the end hardening with hot water.

All the tested seed priming methods highlight different embryo developmental strategies regarding the secondary stages present. The seeds from osmo-hardening treatments had at all the assessments the highest number of BBCH secondary stages present. Then, the following treatments in terms of seed stimulation were hardening with hot water, hydropriming and at the end the seeds from the control treatment with the lowest secondary stages present at the last assessment after 56 days.

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CLIMATIC INPUTS INFLUENCE ON THE YIELDS OF WILD BERRIES HARVESTED FROM TRANSYLVANIAN TESTING SPONTANEOUS FLORA. A CASE STUDY: *RUBUS IDAEUS* L., AND *RIBES NIGRUM* L. IN THE SEASON OF 2022

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Abstract

Even though the influence of the climatic traits on wild berries production and quality is well known, this issue is of interest to be analysed in connection with specific species, areas, and climate. The present study was conducted for characterizing the crude chemical composition, pH, ascorbic acid, and total phenolics and of studied wild berries species, and quantifying the interrelationships among climatic conditions characterizing the fruits collecting area and their dry matter content. In this aim, there were taken into consideration two species of wild berries, Rubus idaeus L. (raspberry), and Ribes nigrum L. (blackcurrants), respectively, harvested from Colibiţa area, Bistriţa-Năsăud County, in the autumn of 2022. The influences of the precipitations, temperature, atmospheric pressure, and wind velocity on studied wild berries dry matter content are quantified. The above-mentioned climatic parameters were collected from databases, and averages were calculated by wild berries vegetation period (March-September 2022). According to the multiregression analysis, and simple correlations calculation, only precipitations and temperature influence the wild berries dry matter content. The studied interrelationships show that increasing trends in temperature and rainfall regimen are moderate and positively correlated with both fresh and dry yields of raspberry, and blackcurrants.

Key words: *blackcurrant, multiregression analysis, parameter, raspberry, vegetation period.*

INTRODUCTION

It is well known that both wild and cultivated berries are a rich source of bioactive secondary metabolites, fibers, vitamins, sugars, phenolics, or polyunsaturated fatty acids.

Numerous studies have shown their significant beneficial effects on human health (Nile and Park, 2014).

Blackcurrants (*Ribes nigrum* L.) native to Northern and Central Europe also North Asia is considered a rich source of nutritional and bioactive compounds.

Literature shows that they represent a very rich source of phenolics (Ovaskainen et al., 2008). The blackcurrants seeds are used as raw material for oil extraction, which is widely used in cosmetics (Lu and Foo, 2003; Matilla et al., 2006).

Raspberries (*Rubus idaeus* L.) is considered native from areas that now are part of the Turkish state, but today is spread worldwide. The fruits are also rich in nutrients and antioxidants as phenolics, which are present in amounts that varies between varieties (Anttonen and Karjalainen, 2005).

The concentration of the bioactive compounds contained in wild berries, including raspberry, and blackcurrants, are known to be influenced by environmental factors (Anttonen and Karjalainen, 2005; Kaldmäe et al., 2013; Remberg et al., 2010).

The present study was conducted for characterizing the crude chemical composition, pH, ascorbic acid, and total phenolics and of studied wild berries species, and quantifying the interrelationships among climatic conditions characterizing the fruits collecting area and their dry matter content. In this aim, there were taken into consideration two species of wild berries, *Rubus idaeus* L. (raspberry), and *Ribes nigrum* L. (blackcurrants), respectively, harvested from Colibiţa area, Bistriţa-Năsăud County, in the autumn of 2022.

MATERIALS AND METHODS

The raspberry and blackcurrants are the wild berries considered in this study. They were collected from Colibița area, Romania (47°10′14″N 24°53′17″E), in the autumn of 2022. Data concerning the climatic inputs (precipitations, environmental temperature, atmospheric pressure, wind velocity) were collected from specialized databases, (www.meteoblue.ro, https://www.wunderground.com/history /monthly/ro/).

Fruits were conditioned for the chemical analysis in laboratory. The crude chemical composition (water, dry matter, crude protein, crude dietary fibre, crude ash, and nitrogen free compounds) was determined using the Weende scheme (Șara and Odagiu, 2002). For determination of ascorbic acid and total phenolics contents the fruit samples were homogenized with a blender and extracted four times with Ethanol:Water (20:80 v/v) in a 1:3 v/v homogenate:solvent ratio in the dark (15 min each sample).

The total phenolics content was determined by the Folin-Ciocâlteu method as modified by (Singleton et al., 1999). Ascorbic acid was quantified by the Karlsen et al. (2005) methodology, modified by Aaby et al. (2007). Statistical analyses were performed using the software STATISTICA v.12,0 for Windows (StatSoft).

Basic statistics was applied to raw data in order to calculate the means of the crude chemical composition of the fruit's samples, pH, ascorbic acid, and total phenolics.

To calculate the simple correlations between the dry matter content and considered climatic inputs because the linearity of the dependence between variables was not identified, we calculate the correlation using the Spearman non-parametric methodology.

Multiple correlations were calculated between climatic inputs (precipitations and environmental temperature) strong and moderate correlated with fruits dry matter content.

RESULTS AND DISCUSSIONS

During the wild berries vegetation period the average amount of precipitation was of 2.64 mm, which corresponds to a sum of 592 mm, and average temperature was of 13.89°C. In 2022, during climate parameters monitoring period, the experimental area was characterized by low atmospheric pressure (with an average of 734.57 mm Hg), and wind velocity (with an average of 2.86 m/s). Except atmospheric pressure, high dispersion values are observed for the other climatic parameters. This suggests their high variability which may be explained for temperatures, and precipitations by the succession of three seasons – spring, summer, and beginning of autumn (Table 1).

A mean dry matter of 12.59% is reported for the raspberry fruits collected from Colibița area. Expressed from dry matter the nutrients identified as having the largest mean share are the crude dietary fiber (56.12%), and nitrogen free compounds (27%). The dry matter mean is within the rages observed by Skrede et al. (2012), and Mazur et al. (2014) in cultivated raspberry, but the mean protein content is double, compared to the value reported by Koraqi et al. (2019). For the dietary fiber content we report a higer mean compared to the range (5.20-6.34%) reported by de Souza et al. (2014).

The mean pH recorded the value of 2.81, while for ascorbic acid and total polyphenolics are reported the mean values of 1921.03 mg/100 g dry matter, and 259.09 mg GAE/100 g dry matter. The pH, ascorbic acid, and total polyphenolic means are within the rages observed by Mazur et al. (2014) in ten raspberry cultivated genotypes.

Compared to the pH and total phenolics means reported in 3 wild ecotypes from Turkey by Gülçin et al. (2011) ranging between 3.65 – 3.70, and 91.6-231 mg GAE/100 g dry fruit, the means reportd in our study emphasizes lower value for pH, but much higher for the total phenolics.The crude protein and nitrogen free compounds presented high variability, 29.53%, and 29.70%, respectively (Table 2).

Compared to raspberries, the blackcurrants collected from the same experimental area have a higher mean dry matter content, of 17,36%. The dry matter mean is under the rages (18.90-20.07%) observed by Oancea et al. (2011), in wild blackcurrants harvested from Brașov area, Romania, and by Marjanovic-Balaban et al. (2012) in Serbia (19.54-24.94%).

Table 1. The average evolution of the climatic parameters in Colibița area by March-September 2022

Issue	n	Mean	Sum	Min.	Max.	Stand.dev.
Precipitations (mm)	214	2.64	592.00	0.00	24.00	4.13
Temperature (°C)	214	13.89	33191.00	-1.00	26.00	6.18
Atmospheric pressure (mm Hg)	214	734.57	112389.92	718.82	751.84	5.30
Wind velocity (m/s)	214	2.86	436.91	0.20	15.00	2.60

Table 2. The crude chemical composition (%), water (%), dry matter (%), pH (pH units), ascorbic acid (mg/100 g fruit) and total phenolics (mg GAE/100 g fruit) in raspbery fruits colected from spontaneous flora of Colibița area, in 2022 season

Issue	n	X[9]		Min.		Max.		s[10]		CV%[11]	
		FM[8]	DM	FM	DM	FM	DM	FM	DM	FM	DM
Water	30	87.41	-	85.00	-	90.00	-	1.66	-	1.89	-
DM[1]	30	12.59	-	10.00	-	15.00	-	1.66	-	13.15	-
CP[2]	30	1.42	11.26	0.90	7.15	2.10	16.68	0.42	3.33	29.53	29.53
CF[3]	30	7.07	56.12	6.10	48.45	8.00	63.54	0.54	4.32	7.70	7.70
CA[4]	30	0.71	5.62	0.59	4.69	0.81	6.43	0.07	0.59	10.52	10.52
NFC[5]	30	3.40	27.00	0.13	1.03	6.70	53.22	1.01	8.01	29.70	29.70
pH	30	2.81		2.59		2.95		0.21		7.47	
AA[6]	30	241.91	1921.03	230.05	1827.24	249.62	1982.68	19.39	154.01	8.01	8.01
TP[7]	30	32.62	259.09	27.55	218.82	40.26	319.78	2.95	23.43	9.04	9.04

[1]DM - dry matter; CP[2] - crude protein; CC[3] - crude dietary fiber; CA[4] - crude ash; NFC[5] - nitrogen free compounds; AA[6] - ascorbic acid; TP[7] - total phenolics; FM[8] - fresh matter X[9] - mean; s[10] - standard deviation; CV[11] - coefficient of variation.

Compared to raspberries, the blackcurrants collected from the same experimental area have a higher mean dry matter content, of 17.36% (Table 3). The dry matter mean is under the rages (18.90-20.07%) observed by Oancea et al. (2011), in wild blackcurrants harvested from Brașov area, Romania, and by Marjanovic-Balaban et al. (2012) in Serbia (19.54-24.94%). The same, expressed from dry matter, the nitrogen free compounds (82.85%) were identified as having the largest share, while the other nutrients quantified recorded averages between 8.60% crude protein - 3.95% crude crude dietary fiber. pH recorded a mean of 3.02, ascorbic acid a mean of 1445. 27 mg/100 g dry matter, and total phenolics a mean value of 276.09 mg GAE/100 g dry matter. Our study emphasizes protein, ash and ascorbic acid blackcurrants contents within the ranges reported by Marjanovic-Balaban et al. (2012) in Serbia, (1.42-1.65% fresh matter, and 0.68-1.10% fresh matter, 1.9-60.51 mg/100 g fresh fruit, respectively).

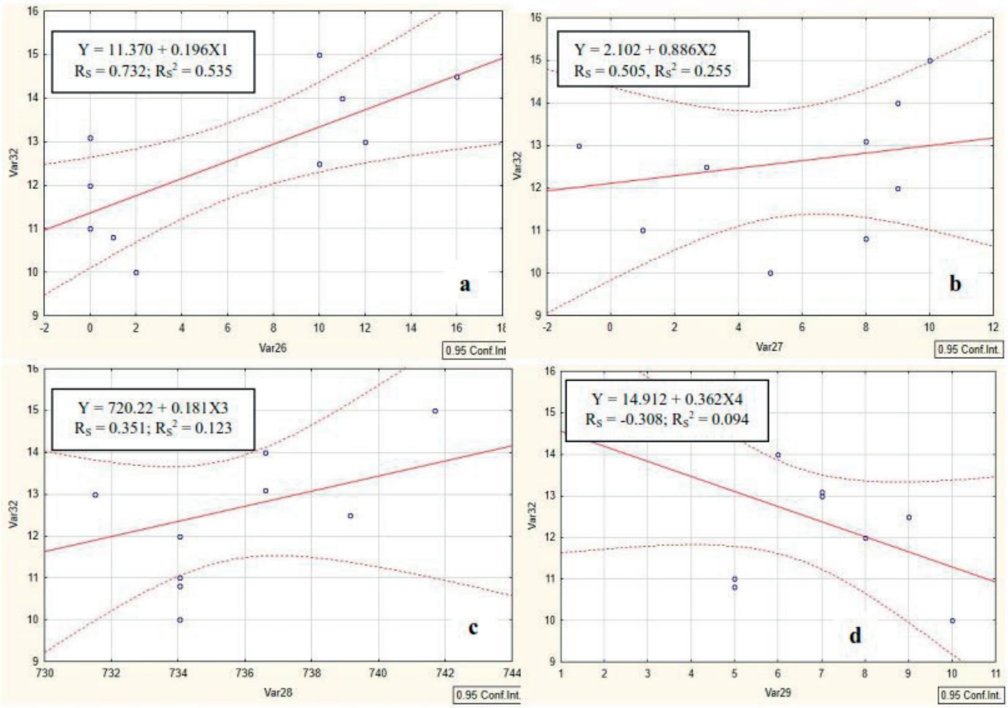
Compared to the total phenolics means reported in 4 wild ecotypes from Argentina and Chile by Jiménez-Aspee et al. (2015) ranging between 48-320 mg GAE/100 g fresh fruit, and and 2 ecotypes from Portugal by Spínola et al. (2019) ranging between 87.20.6-103.42 mg GAE/100 g fresh fruit, the mean reportd in our study is lower. The mean pH determined in our study is within ranges 2.92-3.17, reported in 8 cultivars from Canada by Zatylny et al. (2004). Unlike rasperries, for all nutrients low variabilities, under 11% are reported (Table 3).

Table 3. The crude chemical composition (%), water (%), dry matter (%), pH (pH units), ascorbic acid (mg/100 g fruit) and total polyphenolics (mg GAE/100 g fruit) in blackcurrants fruits colected from spontaneous flora of Colibița area, in 2022 season

Issue	n	X		Min.		Max.		s		CV%	
		FM[9]	DM	FM	DM	FM	DM	FM	DM	FM	DM
Water	30	82.64	-	80.80	-	84.60	-	1.33	-	1.61	-
DM[1]	30	17.36	-	15.40	-	19.20	-	1.33	-	7.68	-
CP[2]	30	1.49	8.60	1.39	8.01	1.60	9.22	0.07	0.43	4.99	4.99
CC[3]	30	0.69	3.95	0.59	3.40	0.80	4.61	0.07	0.42	10.74	10.74
CA[4]	30	0.80	4.60	0.70	4.03	0.90	5.18	0.06	0.35	7.70	7.70
NFC[5]	30	14.38	82.85	12.51	72.06	16.21	93.38	1.39	7.99	9.65	9.65
pH	30	3.02		2.95		3.14		0.23		7.61	
AA	30	25.09	1445.27	23.45	1350.81	27.02	1556.45	2.23	12.84	8.89	8.89
TP	30	47.93	276.09	45.00	259.21	48.00	276.49	2.26	13.01	4.71	4.71

[1]DM - dry matter; CP[2] - crude protein; CC[3] - crude crude dietary fiber; CA[4] - crude ash; NFC[5] - nitrogen free compounds; AA[6] - ascorbic acid; TP[7] - total phenolics; FM[8] - fresh matter X[9] - mean; s[10] - standard deviation; CV[11] - coefficient of variation.

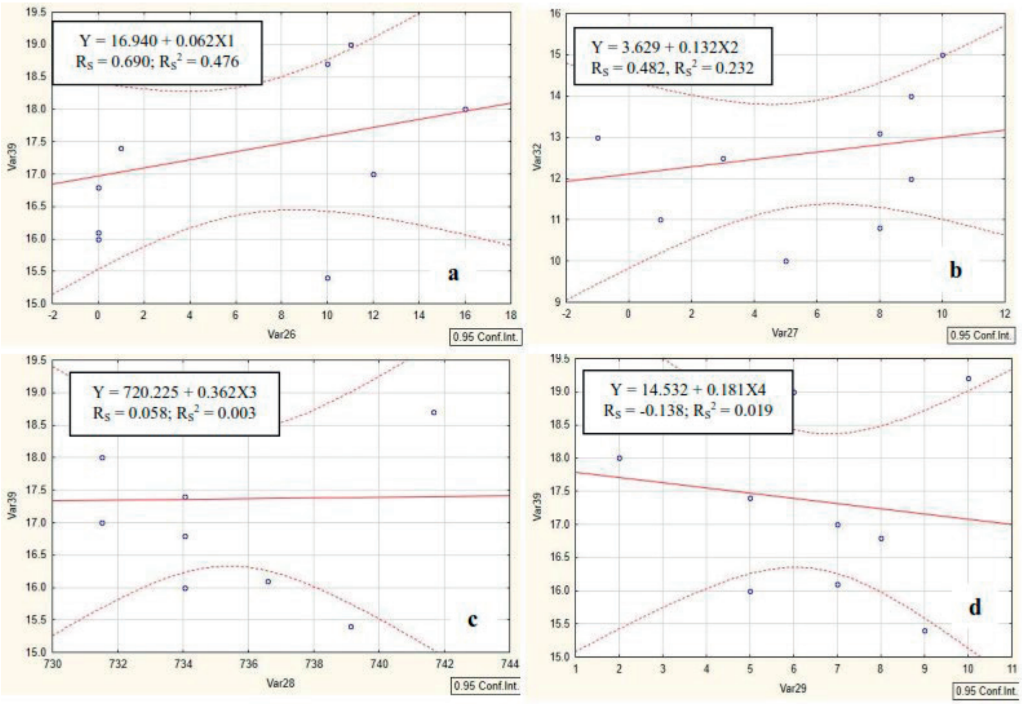
Between raspberries dry matter content and precipitations the strong correlation (R = 0.732) identified shows that in 53.50% of cases the increase of the rainfall regimen is accompanied by the increase of dry matter content in a manner depicted by the regresion line, Y = 11.370 + 0.196X (Figure 1a).
The same evolution is also observed for the correlations between raspberries dry matter content and environmental temperature, or atmospheric pressure, mentioning that if concerning the environmental temperature it has a mean intensity (R = 0.505), while with atmospheric pressure, according to the value of the correlation coefficient (R = 0.351) is may be framed as weak (Figure 1b, and Figure 1c).
Mazur et al. (2014) found positive correlations between a raspberry cultivar fruits weight at harvesting and a week before harvesting, and environmental temperature, ranging between R = 0.460-0.530. Studies performed in Norway by, published in 2014 and 2016, emphasized positive correlations ranging between R = 0.380-0.800, between dry matter of five blackcurrants cultivars, and environmental temperature, while between above mentioned cultivars and precipitations, unlike our findings, the results are controversial, meaning that in the study published in 2016 they were negative, ranging between R = 0.090-0.640, while in the study published in 2015 they were both positive and negative, function of cultivar.
A negative weak correlation (R = -0.308) is reported between raspberries from spontaneous flora dry matter content and wind velocity and this shows that in only 9.40% of cases the increase of the wind velocity corresponds to the increase of dry matter content in a manner, which is suggested by the regresion line, tha also shows by the 0,094 coefficient the low contribution of the wind velocity Y = 14,912 + 0.094X (Figure 1d).



Var 26 - precipitations (mm); Var 27 - precipitations (°C); Var 28 - atmospheric pressure (mmHg); Var 29 - wind velocity (m/s); Var 32 – raspberries dry matter production (g); Y - raspberries dry matter production; X1 - precipitations; X2 - temperature; X3 - atmospheric pressure; X4 - wind velocity; R_s - coefficient of correlation Spearman; R_S^2 - coefficient of determination.

Figure 1. The simple correlation between the climatic factors precipitations (a), temperature (b), atmospheric pressure (c), wind velocity (d), and raspberry dry matter

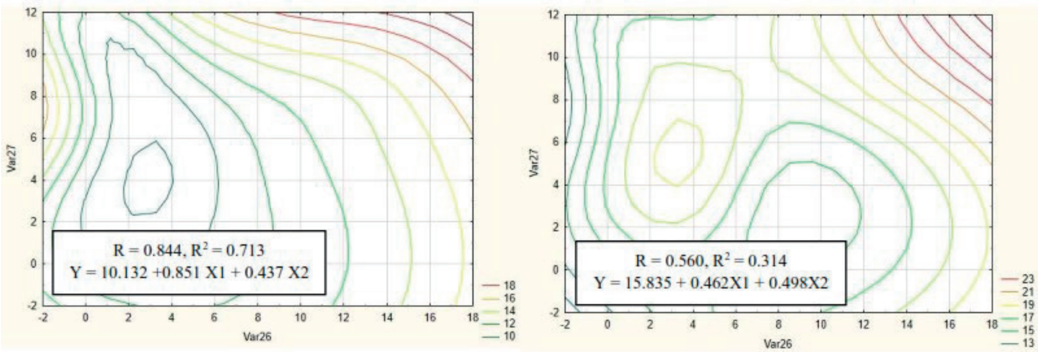
Concerning blackcurrants, also strong correlation (R = 0,690) is identified between dry matter content and precipitations, and this emphasizes that in 47.60% of cases the increase of the rainfall regimen is accompanied by the increase of dry matter content in a manner depicted by the regresion line, Y = 16.940 + 0.062X (Figure 2a). Studies performed in Norway by Woznicki et al., published in 2015 and 2016, emphasized positive correlations ranging between R = 0.380-0.800, between dry matter of five blackcurrants cultivars, and environmental temperature, while between above mentioned cultivars and precipitations, unlike our findings, the results are controversial, meaning that in the study published in 2016 they were negative, ranging between R = 0.090-0.640, while in the study published in 2015 they were both positive and negative, function of cultivar. With the environmental temperature a mean intensity (R = 0.482) is reported, while with atmospheric pressure (R = 0.058) it is very weak (Figure 2b, and Figure 2c). A negative weak correlation (R = -0.132) is reported between blackcurrants dry matter content and wind velocity and this shows that in only 1.90% of cases the increase of the wind velocity corresponds to the increase of dry matter content in a manner depicted by the regresion line, Y = 14,532 + 0.181X (Figure 2d).



Var 26 - precipitations (mm); Var 27 - precipitations (°C); Var 28 - atmospheric pressure (mmHg); Var 29 - wind velocity (m/s); Var 36 – blackcurrants dry matter production (g); Y – blackcurrants dry matter production; X1 – precipitations; X2 – temperature; X3 - atmospheric pressure; X4 - wind velocity; R_s – coefficient of correlation Spearman; R_S^2 – coefficient of determination.

Figure 2. The simple correlation between the climatic factors precipitations (a), temperature (b), atmospheric pressure (c), wind velocity (d), and blackcurrants dry matter

According to the values obtained for the simple correlations, only rainfall regimen and temperature affect the wild berries yields.
The studied interrelationships between plants and climatic factors show that increasing trends in temperature and rainfall regimen are positively strong correlated (R = 0.844) with raspberry dry matter production, and positively moderate correlated (R = 0.560) with blackcurrants dry matter production (Figure 3).



a b

Var 26 - precipitations (mm); Var 27 - temperatures (°C); a - raspberry dry matter production (g); b – blackcurrants dry matter production 9g); X1 - precipitations; X2 – temperature; R - coefficient of multiple correlation; R^2 - coefficient of determination.

Figure 3. The multiple correlation between the climatic factors precipitations, temperature, and dry matter of raspberry (a) and blackcurrants (b) fruits colected from spontaneous flora of Colibița area, in 2022 season

The study of the multiregression line, in raspberry (Y = 10.132 + 0.852X1 + 0.437X2), shows that the precipitations positively influence in a higher measure the evolution of the dry matter content (Figure 3a), while in blackcurrants (Y = 15.835 + 0.462X1 + 0.498X2) both precipitation and environmental temperature positively influence, in about the same extent, the dry matter content (Figure 3b).

CONCLUSIONS

This study shows that both studied species raspberries, and blackcurrants, respectively have a high content in water of 87.71%, and 82.64%, respectively. In raspberry the highest share of nutrients expressed from dry matter are the crude dietary fiber (56.12%), and nitrogen free compounds (27%), and in blackcurrats nitrogen free compounds (82.85%) and crude protein (8.60%. in influence of the precipitations, temperature, atmospheric pressure, and wind velocity on studied wild berries fresh and dry yields are quantified. The above-mentioned climatic parameters were recorded daily, and monthly averages were calculated during wild berries vegetation period. Both species contain large amounts of total phenolics According to the multiregression analysis, and simple correlations calculation, only rainfall regimen and temperature affect the wild berries dry matter content, of 259.09 mg GAE/100 g raspberry dry matter, and 276.09 mg GAE/ 100 g blackcurrants dry matter. The studied interrelationships show that increasing trends in temperature and rainfall regimen are moderate and positively correlated with dry yields of raspberry, and blackcurrants. These results show that weather conditions represented by precipitations and environmental temperature have a positive contribution to the dry matter content of raspberry and blackcurrant species from the spontaneous flora, within the specific climate of Colibița area, Romania.

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CHLOROPHYLL CONTENT AND STOMATA MORPHOMETRIC FEATURES OF *ANETHUM GRAVEOLENS* L. IN A CONTROLLED EXPERIMENT WITH DIFFERENT SALINE LEVELS

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Abstract

The intensive use for different purposes of dill requires split and even short-term studies that are desirable in purpose to highlight the threatening thresholds of salinity doses during vegetation growth season. The aim of the study was to assess the dill growth according to BBCH scale, the leaves' chlorophyll content and stomata number, and morphological characteristics during growth and development under salinity stress. The experiment was set under controlled conditions at 20±2°C T, and 40% H under full light in the growth chamber. Two different salinity levels of 50 mM, 100 mM NaCl and a control were tested in 3 treatments for 5 plants in 6 repetitions. Dill germination rate registered the higher value at 50 mM NaCl compared to the control treatment. At the end of the 10 BBCH germination stage, the lowest NaCl dose determined 100% of germination capacity. The higher stomata density was in the treatment with 50 mM NaCl, with 46% more compared to the control. The chlorophyll content of dill decreased with the increasing salinity levels. Dill is not negatively influenced by the two salinity doses tested.

Key words*: BBCH scale, dill, germination, physiological parameters, SPAD units.*

INTRODUCTION

Medicinal and aromatic plants (MAPs) are very important for the economy, being highly used for various purposes like alternative medicine, the cosmetic industry, and most important in global alimentation due to their aromatic scent and also for their antioxidant and relaxing properties (Stoian et al., 2022).

An annual plant, dill *(Anethum graveolens* L.*)* is part of the Apiaceae family. Native from Central and Southeast Asia, but also found in the Mediterranean area of Europe. It is recognized for its qualities as an aromatic and medicinal plant (Kaur et al., 2021). Dill is used to adding flavor to salads, soups, and dishes, but also for its therapeutic qualities, especially against digestive problems. Dill seed essential oil contains carvone, limonene, and alpha-phellandrene (Wall et al., 1986). Along with the other chemical components of the plant, they ensure effects such as antimicrobial, anti-inflammatory (Abdel-Aziz et al., 2016), analgesic, and calming problems of the gastrointestinal tract and in the female reproductive system conditions (Al-Snafi et al., 2014). Together with other aromatic plants, dill could be susceptible to different abiotic stress. One of the most important stressors is considerend salinity stress which intensifies in large areas globally in the current context of climate change.

Salinity is an abiotic stress that represents a threat to agriculture, inhibiting and prohibiting the plant's physiological mechanisms (Yadav et al., 2020). Saline stress restrains the proper nutrition of the plant, a fact that ultimately leads to the defective production of secondary metabolites, the main components of the MAPs essential oils (Ghassemi-Golezani et al., 2022). Essential oils represent a standard in the assessment of this group of plants quality (Li et al., 2020). Even though salinity can delay or limit the development of aromatic plants, there are also species of medicinal plants that manage to grow in an environment with low salinity, such as *Achillea millefolium* and *Matricaria chamomilla* (Máthé et al., 2015). Although MAPs are intensively used both in food and in alternative medicine, being the first treatments used by humans (Marshall, 2011), in Romania, their vegetable agricultural production decreased from 20.459 tons in 1990 to 3.313 in 2021 (INSSE, 2021). In the climate change context, drought, and intensive use of irrigation, soil salinization is a serious consequence that has a various series of effects on the growth and development of plants (Okur et al., 2020). The establishment of osmotic stress caused by water deficit and nutritional imbalance are followed by adaptation mechanisms of plants, including the accumulation of proline (Heuer, 2010), proteins, and soluble carbohydrates (Pirzad, et al., 2011). It can also lead to a decrease in the level of phenolic content, therefore reducing the antioxidant potential of plants. These biochemical imbalances lead to a reduction in the intensity of photosynthesis, the closing of the stomata, and a decrease in the seed germination rate (Chaves et al., 2009). Regarding medicinal plants, the most important physiological process is precisely germination, a process initiated by germplasm activation (Moldovan et al., 2022) and is strongly influenced by the soil salinity. Plants have different degrees of salinity tolerance, and aromatic and medicinal plants are largely resistant to decreased levels of salinity. The concern is the influence of saline stress on the level and composition of secondary metabolites biosynthesized. There is a fine line between benefits and toxicity, reason why it is important to correctly classify plants according to the salt resistance degree and to identify the salt maximum dose that can redefine medicinal plant into a toxic one. Some studies mention *Melissa officinalis* and *Cuminum cyminum* as sensitive to salinity by decreasing the germination rate and percentage (Younesi and Moradi, 2014). According to Mathe, *Achillea millefolium* and *Matricaria chamomilla* are plants resistant to low levels of salinity (Máthé et al., 2015). On the contrary, Said-Al Ahl places *Ocimum basilicum* and *Matricaria chamomilla* in the category of plants sensitive to salinity, the stress-inducing the defective mobilization of reserve substances and suspending cell division (Said-Al Ahl and Omer, 2011). *Ocimum majorana* records in the presence of 100 mM NaCl a decreased essential oils content (Li et al., 2020). *Coriandrum sativum* showed a decrease only in the case of very high values of NaCl, and an impressive increase in essential oils was found at 25-50 mM NaCl (Li et al., 2020). Even though salinity can delay or limit the development of aromatic plants, there are also species of medicinal plants that manage to grow in an environment with salinity, such as citronella *Cymbopogon winterianus* and lemongrass *Cymbopogon citrates* (Aishwath and Lal, 2016).

Previous studies on dill *Anethum graveolens* salt tolerance and how its physiological processes are affected are few, the plant was included in the group of plants with resistance to decreased levels of salt (Aishwath and Lal, 2016). On the contrary, Soliman and Abou-Ellail reported that dill germination is not influenced by salinity, but during the early growth period, the plant is very sensitive to the saline stress (Soliman and Abou, 2016). Reason of which, the aim of the study was set to test de dill growth according to BBCH scale. Hereby were assessed the leaves' chlorophyll content and stomata number, together with morphological characteristics during growth and development under salinity stress.

MATERIALS AND METHODS

The experiment was conducted under controlled conditions. A growth chamber was used with the following constant properties: intensive light from 20 of 18 W neon lights, humidity of 40% and 20±2°C temperature. The tested treatments have consisted of a number of three variants respectively 50 mM NaCl, 100 mM NaCl, and 0 mM NaCl as control, in six replications for 28 days. Saline doses in 50 ml water were applied every three days during watering time. Dill *Anethum graveolens* L. seeds were purchased from Dr. Soil GmbH Germany. Previously to the experiment, the seeds were sterilized using a 50% ethanol solution for 15 minutes in circular moves, so that the seeds flote above solution and then were rinsed for three times with 100 ml distilled water (Lindsey et al., 2017). All the viable seeds were placed in 25 g soil. The soil substrate had a pH between 5-6.5 and a N content<1.9% in 150-250 mg/l; $P(P_2O_5)$ <0.3% in 100-200 mg/l; $K(K_2O)$ <0.7% in 200-300 mg/l from AGRO CS, Romania. During the experimental time, the number of germinated seeds was daily assessed together with the seed development stage on BBCH scale. This scale represents the newest standardized growth and development monograph for plants (Meier, 2018). Here are comprised principal and secondary growth stages from the vegetation period of a plant.

According to Sedghi et al., germination percentage (**GP**) was calculated as the proportion of viable seeds in a given population, and is determined by dividing the number of seeds ($\boldsymbol{n}$) that have successfully germinated by the total number of tested seeds ($\boldsymbol{n\,T}$) (Sedghi et al., 2010).

$$\mathrm{GP}\ \% = \boldsymbol{n}/\boldsymbol{n\,T} \times 100$$

The germination rate **GR** was established for each treatment in the day where around half of tested seeds succeed to germinate and it was calculated as a percentage (Liopa-Tsakalidi et al., 2011).

Chlorophyll content was measured using the MC-100 S/N Apogee Instruments chlorophyll meter, in SPAD units for each plant at the end of the experiment. This is a non-destructive method and gives accurate measurement of instant values. Before each read, the chlorophyll meter was calibrated and the values represent absolute µmol m^{-1}.

Stomata imprints were collected at the end of the experiment. The leaf adaxial surface was covered with red nail varnish then after preliminary drying, sticky tape was used to peel the leaf imprint and placed under a microscope glass slide. The slide prints were assessed under the microscope Olympus CX43 and the images were captured with camera model PROMICAM PRO4K of 12 MP.

The database was analyzed with RStudio software, Anova and Fisher LSD were performed at $p<0.05$ for dill leaves chlorophyll content under salinity treatments in SPAD units, the parameter chlorophyll content was presented as average data ± standard error (S.E.).

Percentage values were projected for germination rate and for BBCH secondary stages distribution also on all performed assessments. Respectively for the other parameters like stomata aperture and density were presented average values and standard deviation (S.D.) on the figures.

RESULTS AND DISCUSSIONS

The most favorable treatment for dill germination was at 50 mM NaCl. In the first germination assessment (D1), after two days' germination capacity values of 100% were recorded in two of the sixth repetitions (Figure 1). On the second assessment of the experiment (D2 after 4 days), maximum germination capacity was recorded in all variants with 50 mM NaCl tested.

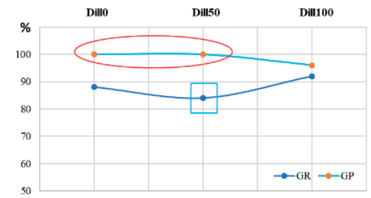

Figure 1. Dill germination rate (GR) and capacity (GP) in percentages under salinity stress (Dill 0-control, Dill 50-50 mM NaCl, Dill 100-100 mM NaCl)

On average, dill seeds from the 100 mM NaCl treatment showed a very similar trend to the control.

The maximum germination capacity of dill seeds of the control variant was reached after eight days compared to 50 mM NaCl variant which registered the maximum germination capacity on the fourth day. The time to reach the maximum germination percentage with 50 mM NaCl Dill 50 treatment was 4 days, reduced by 3 days compared to the same dose applied in the Liopa-Tsakalidi et al (2011) study.

Dill seeds from the 100 mM NaCl treatment had a germination capacity of 96% on the sixth day of the experiment. This percentage did not change until the end of the experiment, probably due to the fact that for 4% of the dill seeds, the saline dose showed a slightly toxic, germination-inhibiting effect.

In the case of the control and the 100 mM NaCl treatment, four out of six replicates were represented by seeds with 100% germination capacity. The germination percentage is not affected by the reduced saline dose. The high NaCl dose (100 mM Dill 100) can affect PG, which was also proven by previous studies by Ravender and Kundu (2000). According to Liopa-Tsakalidi et al (2011) study, the application of a dose five times higher than the highest dose in our study (100 mM NaCl) inhibited the germination process of 11 MAPs species, of which, one was the dill. Similar to a study conducted in 2011 (Liopa-Tsakalidi et al., 2011), the dill seeds germination in our study was only slightly affected by a concentration higher than 50 mM NaCl, i.e. 100 mM NaCl. Similar to the Liopa-Tsakalidi et al. studies (2011), the germination percentage at the most concentrated dose tested in our study was not significantly affected. The studies (Liopa-Tsakalidi et al., 2011) mentioned above report that only concentrations from 500 mM NaCl affect germination.

The seeds germination rate subjected to salinity stress differed according different tested dose. At the 50 mM NaCl treatment, the germination rate for dill was with 12% higher than in the control variant. For seeds subjected to 100 mM NaCl, this process was reduced three times with a difference of 4% higher compared with the control. Contrary to what was reported in a 2000 study (El-Darier and Youssef, 2000) on the germination rate of cress seeds, the highest GR value in our study was recorded at the double saline dose respectively at 100 mM NaCl Dill 100.

The resistance of the germination process to salinity varies depending on the plant and its variety, but also on the salt stress intensity. Under high salinity conditions, the germination percentage and rate can be enhanced by applying GA_3 which alleviates the harmful effects of sodium accumulation (Sedghi et al, 2010).

The most higher dill seed percentage in BBCH 09 - emergence - cotyledons breaks through soil surface, at the first assessment (D1) was in the treatment with 50 mM NaCl (Dill 50) (Figure 2). This percentage was higher that the control with 13% and the most lower seeds percent of 23% in this secondary stage was in the treatment with 100 mM NaCl (Dill 100) compared with the control.

At the second assessment (D2) after 4 days, the seeds followed the same trend as in the previous assessment only regarding the maximum percentage value of the Dill50 treatment, the seeds reached BBCH 09 in the same percentage of 90% in the control and Dill100 treatments.

At the third assessment (D3) after 6 days it was observed an accelerated development of secondary stage at the treatment Dill 100 completely into BBCH 10 represented by completely unfolded cotyledons with 3% higher value compared with the previous assessment. Dill 50 was represented by seeds in BBCH 10 in the most lower percentage with 80% compared with the control. After 8 days (D4), the Dill 50 seeds in the following BBCH secondary stage (BBCH 11 with first true leaf unfolded) represented 24% and had a 7% increase compared with the control also registered a decrease of 21% in Dill 100. In the next assessment (D5), the seed percentage in BBCH 11 in Dill 50 doubled. Therefore, compared to the control variant this value was with 30% higher and compared to Dill 100 was with 37% higher. After this assessment (D5) dill seeds from Dill 50 treatment develop further in secondary stages and register the most higher percentages. The percentage difference (D6-D5) of Dill 50 compared with control had decreased to 19% by 11% and compared to Dill 100 in D5 the difference from D6 increased to 53% by 16%. Approximately the same difference it was observed on D7 assessment between Dill 50 and control variant. The difference between Dill 50 and Dill 100 was similar with the D5 percentage of 37%. Representative secondary stages at D8 for Dill 50 and Dill 100 treatments was BBCH 12 (with 2 true leaves unfolded) and BBCH 11 (with first true leaf unfolded). The control variant has an extra 5% of dill seeds in BBCH 10, an inferior developmental secondary stage. Therefore, D8 it can be considered an important threshold to differentiate the speed of seedlings leaf development. Dill seedling growth length. Dill seedlings growth and development were affected by salt stress at concentrations higher than 50 mM (Liopa-Tsakalidi et al., 2011). More than half (67%) of germinated seeds from Dill 50 were in BBCH 12 with a difference of 21% higher compared to the control and 40% higher than Dill 100. Only after 16 days (D8), dill seeds percentage in BBCH 11 from Dill 100 was the highest (66%) with 20% higher than the control and with 36% higher in comparison with Dill 50.

At the assessment D9, after 18 days, only in Dill50 were seen seeds in BBCH 13 with already 3 true leaves unfolded in a relatively low percentage of 3%. This last secondary stage was observed to the other two treatments only after 6 days after three assessments (D12).
In comparison with control treatment at D9, in Dill 50 were with 23% higher dill seedlings in BBCH 12 and with 14% lower in Dill 100. Secondary stage BBCH 11 was represented by the most higher percentage (53%) in Dill 100 with of difference of 10% from the control and 36% compared with Dill 50. All the tested treatments can be further classified based on development under BBCH secondary stages until the end of the experiment. The most active seeds and leaves of fast growing seedlings were observed in Dill 50, followed by the control treatment and Dill 100. In D12 assessment, both the control and Dill50 treatment had a seedlings frequency percentage of leaves development in BBCH 12 and BBC3 13 similar both on the secondary stages and also between the two treatments tested. So, the control variant overcomes the percentages from 50 mM NaCl in D12 compared with the previous assessments. At the end of the experiment, the seedlings in BBCH 13 under Dill50 registered 25% difference compared with the control and 39% difference compared with Dill 100. This percentage trend seen in D13 was similar with assessment D5. After evaluating BBCH distribution in secondary stages for each day, it is obvious that the level of quantifying each response at salinity exposure it has a high degree of difficulty (Poljakoff-Mayber and Gale, 1975).

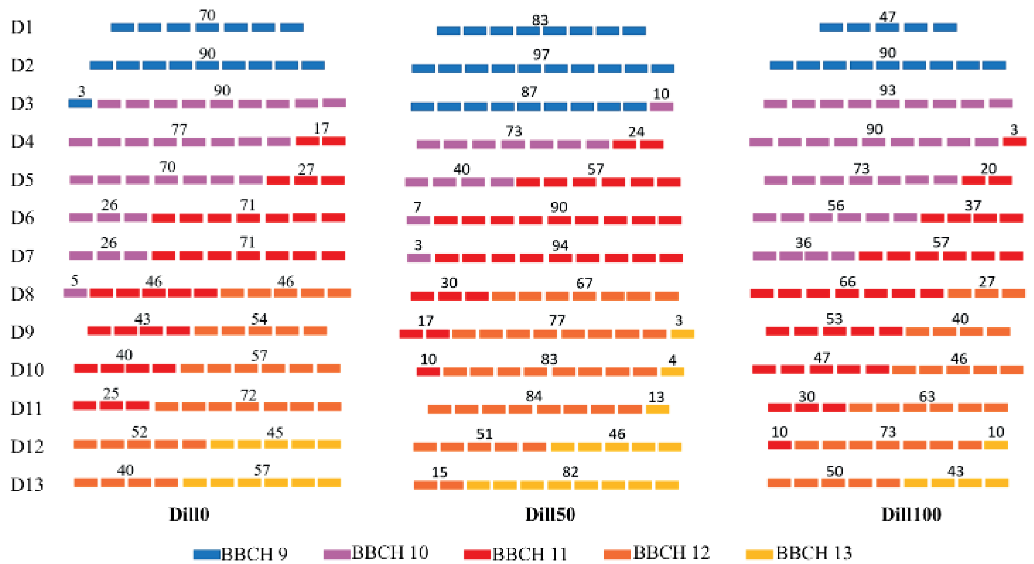

Figure 2. Percentage of germinated dill seeds and seedlings development for each BBCH secondary stage in all assessments (D1, D2…D13) for each treatment (Dill 0-control, Dill 50-50 mM NaCl, Dill 100-100 mM NaCl)

The control variant seedlings recorded the highest chlorophyll content, 44.8±6.0 within a range between 36.9 and 56.5 SPAD units (Figure 3). The low salinity concentration Dill50 seedlings had slightly low value of SPAD units with around 5% compared to the control variant.
The lowest value of chlorophyll content was reported in the high concentration of 100 Mm NaCl around 35.5±0.8 (SE), which registered a decrease of 16.5% compared to the 50 mM NaCl variant and with 21% compared to control.
Contrary to Shekari et al study (Shekari et al., 2017) reporting that under saline conditions at a conductivity of 10ds/m, equivalent to a concentration of about 100 mM (Dheeravathu et al., 2018), respectively the high concentration in our study, chlorophyll levels did not show significant decreases between 0–

50 mM NaCl treatments. In the case of our study, although the tendency of chlorophyll content is to decrease with increasing salt concentrations, the decrease in chlorophyll level between treatments is not significant, contrary to the studies of Hassanpouraghdam et al. (2022). Decreased chlorophyll content may be associated with elevated levels of chlorophyllase enzyme activity (Nikpour-Rashidabad et al., 2016). Other causes of low chlorophyll levels are due to the acceleration of the degradation process of photosynthesis and the decrease of biosynthesis of the essential substance in the photosynthesis process, both processes being consequences of the installation of oxidative stress (Shekari et al., 2017).

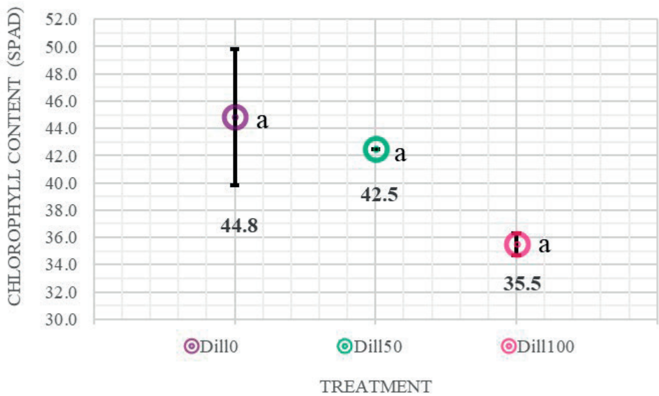

Figure 3. Dill leaves chlorophyll content under salinity treatments in SPAD units (Dill0-control, Dill50-50mM NaCl, Dill100-100mM NaCl). Fisher LSD test, different letters represent significant differences at $p<0.05$.

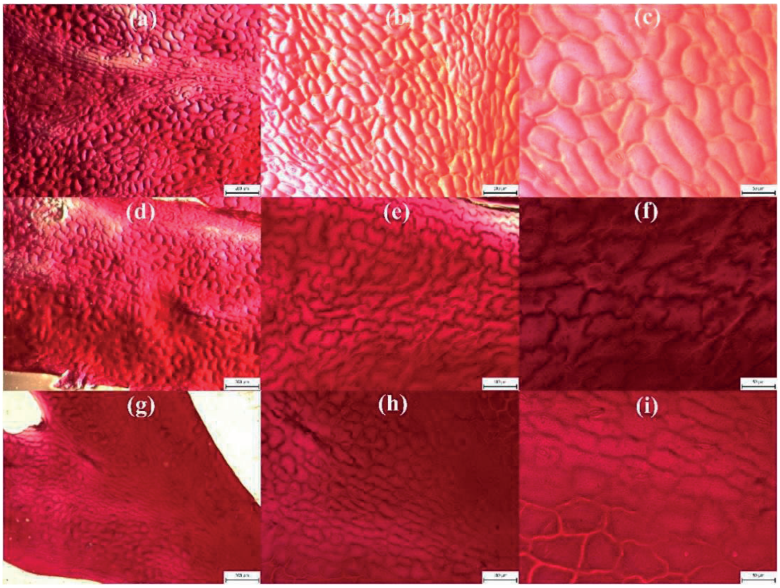

Figure 4. Dill stomata morphometric representative features (a) Dill 0 at 10x-200 μm scale; (b) Dill 0 at 20x-100 μm scale; (c) Dill 0 at 40x-50 μm scale; (d) Dill 50 at 10x; (e) Dill 50 at 20x; (f) Dill 50 at 40x; (g) Dill 100 at 10x; (h) Dill 100 at 20x; (i) Dill 100 at 40x

In the control variant at 10x magnifying (Figure 4, a), the stomata are positioned interspersed along the longitudinal profile of the dill leaf imprint. Their distribution can be characterized as uniform over the leaf surface. The 20-fold magnification image shows details of 5 sides of the annex cells distributed on both sides of the main venation (Figure 4, b). On average, the aperture width is around 25 μm, the cell walls are rounded at the annex cells and have a length of around 50 μm (Figure 4, c). Under the second treatment (Dill 50), stomata have slightly larger aperture than the control (Figure 4, d). Cell walls were altered, perhaps due to salt stress presence, at the lowest dose tested. The cell shape could be characterized by curved lines with 6-9 smooth peaks (Figure 4, e). The doubling of stomatal cells is a counteracting mechanism of saline dose (Figure 4, f). Cells are lax and distorted, placed discontinuously or with large spaces between them compared to the control. Stomata have thickened walls and apertures up to twice as large as the control (Figure 4, g, h, i). The irregular shape of the cells is due to the accumulation of potassium ions in the guard cells. Increased osmotic pressure was proven to be a consequence of ion imbalance installed after salt stress (Wilmer and Fricker, 1996).
In the middle leaf area (Dill 0_M), the average value was the highest 4±1.1, with a maximum of 5 μm stomatal pore aperture or opening between guard cells (Figure 5). On the base of the leaf (Dill 0_B), the average value was 3±0.6 with a minimum of 2 μm.The average adaxial stomata pore aperture on the control (Dill 0_T) leaf tip area was 2±0.6 within the range of 1-3 μm.

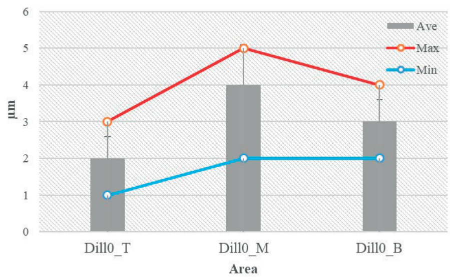

Figure 5. Dill leaves adaxial stomatal pore aperture on control treatment (Dill0_T-pore aperture on leaf tip area, Dill0_M-stomatal pore aperture on middle leaf area; Dill0_B-stomatal pore aperture on leaf base)

For Dill50 the middle leaf area shows the same trend ass Dill 0 (Figure 6). On the middle leaf area (Dill 50_M), the average value was 4.2±0.7, with a maximum of 5 μm stomatal pore aperture. The average stomata pore opening on 50 mM NaCl treatment (Dill 50_T) leaf tip area was 2.8±0.7 within the range of 2–4 μm. On the base of the leaf (Dill 50_B) the average value was 4±1.1 with a minimum of 2 μm.

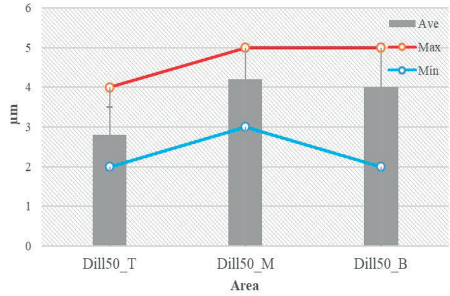

Figure 6. Dill leaves adaxial stomatal pore aperture on 50 mM NaCl treatment (Dill 50_T-pore aperture on leaf tip area, Dill 50_M-stomatal pore aperture on middle leaf area; Dill 50_B-stomatal pore aperture on leaf base)

The adaxial stomata pore aperture on 100 mM NaCl treatment (Dill 100_T) leaf tip area was 4±0.8 within the range of 3-5 μm (Figure 7), double that in the control treatment. In the middle leaf area (Dill 100_M), the average value was 1.8±0.4, with a maximum of 2 μm stomatal pore aperture, twice as low as the Dill 0_M. On the base of the leaf (Dill 100_B), the average value was 1.8±0.7 with a minimum of 1 μm.

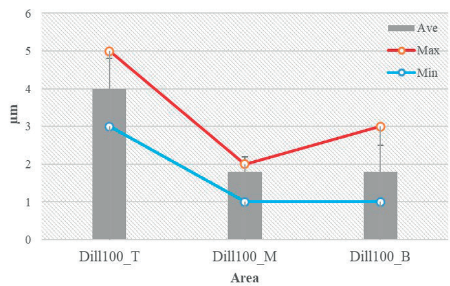

Figure 7. Dill leaves adaxial stomatal pore aperture on 100 mM NaCl treatment (Dill100_T-pore aperture on leaf tip area, Dill100_M-stomatal pore aperture on middle leaf area; Dill100_B-stomatal pore aperture on leaf base)

Compared to the control, the stomatal pore aperture on the tip of the leaf in Dill 50_T is with 40% higher than in Dill 0_T (Figure 5). On Dill 100_T, in the tip of the leaf, the adaxial stomata opening is 100% higher than the control (Figure 6). The average value in Dill 50_M was similar to Dill 0_M (Figure 6). In Dill 100_M the stomatal aperture had decreased by 55% compared to the Dill 0_M value (Figure7). In Dill50_B, the average stomata pore opening was 33% higher than the control Dill 0_B (Figure 6). Dill 100_B registered a decreased average value of the pore opening with 40% compared to the control (Figure 7).

Another similar study, on barley, reported a significant decrease of stomatal pore aperture with the increasing concentration of the saline solution at four treatments (0, 50, 100, 150 mM NaCl) (Hassan et al., 2021). Contrary to this study, our experiment reported a higher average stomatal pore aperture in the 50 mM NaCl variant Dill50 compared to the control variant Dill0. The 100 mM NaCl Dill 100 variant recorded lower average values of stomatal pore aperture compared to the 0 mM NaCl Dill 0 control variant and the 50 mM NaCl Dill 50 variant, similar to the trend reported in Hassan et al., 2021 study. The inhomogeneous appearance of the stomatal pore aperture may be a consequence of sudden changes in environmental factors and is leading to changes in the functioning of the transpiration and photosynthesis processes (Wilmer and Fricker, 1996).

The most highest density of stomata per leaf area in cm^2, was observed in the Dill 50 treatments (Figure 8).

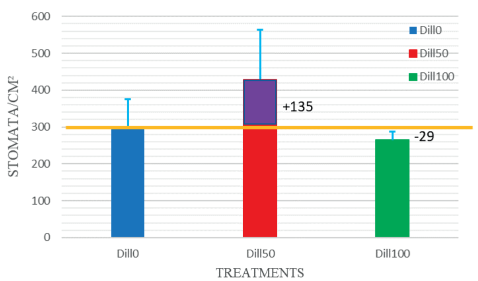

Figure 8. Dill leaves stomatal density (average stomata per total leaf area in number±S.D./cm^2) under salinity (Dill 0-control, Dill 50-50 mM NaCl, Dill 100-100 mM NaCl)

Compared with the control variant Dill 0, this value is with a number of 135 stomata more. The higher salinity dose from Dill 100 had a negative effect of the stromata number, therefore this was lower with 29 compared with the control.

The largest leaf area was recorded for the Dill50 version, i.e., 730 mm^2. The smallest leaf area was recorded for the control variant Dill 0 amounting to 400 mm^2. With a similar value, the leaf area of Dill 100 represents an area of 450 mm^2.

The highest stomata density value recorded for Dill 50 was 429±134 (S.D.) stomata/leaf area and was represented by a 46% increase compared to the stomata density value of Dill 0 (294±81 stomata/leaf area). The lowest stomata density at leaf area level was recorded for Dill 100, i.e. 265±23 stomata/leaf area with 10% lower compared to control - Dill 0.

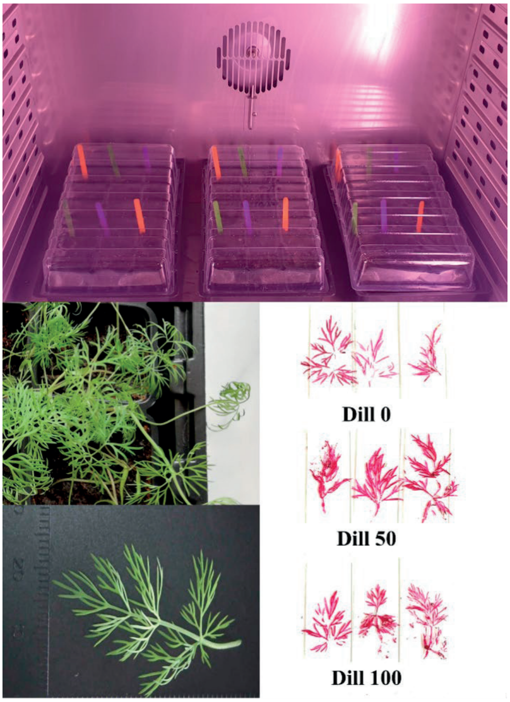

Figure 9. Dill under salinity stress experiment in growth chamber, leaves and leaves imprints for assessment of stomata features

On barley, the stomatal density increases significantly with increasing salt concentration (Hassan et al., 2021). A trend similar to that reported in this study is manifested by dill at the 50 mM NaCl Dill 50 variant. In contrast, the 100 mM NaCl Dill 100 variant recorded a decrease in stomatal density compared to the 0 mM NaCl Dill 0 control and to the 50 mM NaCl Dill 50 variant, contrary to the previously mentioned study.

CONCLUSIONS

The fastest and highest germination capacity value was recorded for the 50 mM NaCl variant on day 4.

The highest germination rate value was also recorded on day 4 but at the 100 mM NaCl variant.

The 0 mM NaCl control variant recorded the highest chlorophyll content expressed in SPAD units. However, chlorophyll levels did not vary significantly with increasing salt concentration.

At 50 mM NaCl concentration, the Dill 50 variant showed high values of germination percentage, stomatal pore aperture, leaf area and stomatal density, indicating a high tolerance of the plant to this level of salt stress.

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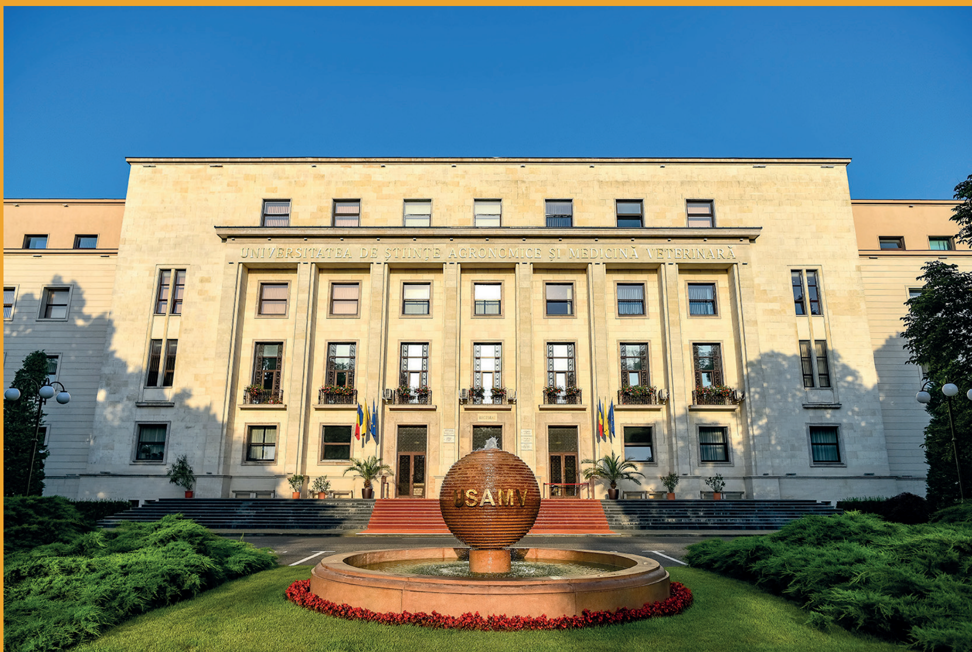

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