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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

FRUIT GROWING

EX·TERRA·AVRVM

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ALMOND, PRESENT AND PERSPECTIVE IN ROMANIA - A REVIEW

Mihaela BĂLUȚĂ, Florin STĂNICĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: miha.baluta@yahoo.com

Abstract

The almond tree (Amygdalus communis L.) is a fruit species of great importance, which finds favourable growing conditions in certain areas in Romania and stands out due to the many properties of its fruit which have a long shelf life. Being a species that enjoys a real appreciation due to its nutritional and medicinal properties, shows interest to fruit growers, making necessary providing some varieties with late flowering and the adoption of modern culture technologies and the aim of this review is to present the current situation and to highlight the importance of relaunching the almond culture in Romania.

Key words*: Amygdalus communis, importance, late flowering varieties, cultivation technologies.*

INTRODUCTION

The almond tree (*Prunus amygdalus* L. sin. *Amygdalus communis* L.) belongs to the family *Rosaceae*, subfamily *Prunoidae*, group *Amygdaleae* and has its origins in arid mountainous regions of Central Asia (Grasselly, 1976a). Almonds were collected here in the wild 10,000 years ago, and they were among the first plants to be domesticated all over the world around the third millennium BC (Albala, 2009). Almonds are considered as one of the oldest crops (Wani et al., 2021).

It is a harsh climate-adapted species with the ability to develop a deep root system in a centrifugal manner, these characteristics allowing the almond tree to explore a wide range of ecological niches, being adapted to conditions with mild, dry winters, warm summers, typical of the Mediterranean climate (Cociu, 2011). The almond is cultivated currently in more than 50 country (Lopez-Granados et al., 2019).

Almond crop has begun to expand in countries with less favorable conditions, and this fact is due because in the last decades a series of new cultivars have been created and cultivation technologies have been improved (Cociu, 2006). This review is about the current state of almond in Romania and the benefits that this crop can bring through the introduction of late flowering varieties and the adoption of modern cultivation technologies.

Almonds became a “luxury” fruit, being exported in large quantities in most advanced countries at a great selling price (Gavăt et al., 2013). Almonds have long been known as a source of essential nutrients; at present, they are in demand as a healthy food with a growing popularity for the general population and farmers. Studies on the composition and characterization of almond macronutrients (Table 1) and micronutrients (Table 2) have shown that the almond has many nutritious ingredients (Barecca et al., 2020; Grundy et al., 2016).

Table 1. Almonds macronutrients

Macronutrients (g)	100 g Fw
Protein	16-23
Lipid	44-61
Saturated Fats	3-4
Monounsaturated Fats	31-35
Polyunsaturated Fats	11-12
Carbohydrates	100 g Fw
Total Sugars	4-6
Total dietary Fibres	11-14

Sources: Barecca et al., 2020; Grundy et al., 2016

Table 2. Almonds micronutrients

Micronutrients (mg)	100 g Fw
Calcium	264-300
Magnesium	230-268
Phosphorus	440-510
Potassium	705-730
Zinc	3.0-4.1
Copper	0.9-1.3
Manganese	1.2-1.8
Vitamins	100 g Fw
Riboflavin	1.0-1.1
Vitamin E	25-27
Total Phenolic Compounds	260-350

Sources: Barecca et al., 2020; Grundy et al., 2016

Almonds is not appreciated only for fresh consumption, but also for their numerous ways of use: in food industry; in chemical-pharmaceutical industry (extraction of a valuable oil); the endocarp, the hard shell, can be used to prepare activated charcoal, necessary for the absorption of toxic gases; the green covering the mesocarp, after drying and calcination, gives an ash that contains 40% potassium and can be used to make soaps and for chemical fertilizers.; Almond wood, due to its color, large density and bright luster after sanding, can be used to make art objects (Cociu, 2003).

The purpose of this review is to emphasize the advantages of cultivating this species in Romania, the importance of introducing new cultivars with late flowering and modern technologies.

MATERIALS AND METHODS

This review is a research based on the current situation of almond crop, the prospective of new cultivars introduction with late flowering and modern cultivation technologies namely: the right choice of planting density and the importance of pruning.

THE CURRENT SITUATION IN ROMANIA

In Romania, the almond tree spreading area is almost overposed with that of vineyard, peach and apricot trees. At the beginning, almond trees could be found intercalated around vineyards, alleys and roads and even in the vineyard because they do don't produce big shadow and doesn't affect vineyard development (Cociu, 2003).

The almond (*Amygdalus communis* L.) being a specific plant to semi-steppe conditions, widely spread in Central Asia, it grows in Romania mainly in Banat, Dobrogea, in the area of vineyards and in many other localities with a warm climate (Ghena et al., 2004).

According to FAOSTAT in Romania there are no statistics of the areas occupied by the almond culture, this crop being included along with the other nut trees.

It should be noted that the interest in this crop is still low in Romania, while the interest shown worldwide is increasing. The almond being one of the crops that lends itself to pedoclimatic conditions in certain areas of Romania, it can be fully mechanized and is accompanied by a multitude of benefits, proving to be an adaptable and versatile species (Albala, 2009).

During the last 30 years, the almond tree has been systematically cultivated in some research stations (Gîtea, 2013).

In order to stabilize the optimal culture areas and the corresponding assortment, experimental and production plots were organized in the Fruit Research Stations in Oradea, Mehedinți, Mărculești and Constanța (Cociu, 2003).

The first cultivar collection was organized in 1951, in Mărculești and after 10-12 years it was transferred at Research Station for Fruit Growing (RSFG) Constanța. In 1975, it was reorganized and doubled at RSFG Oradea (Cociu et al., 2006).

The Romanian gene bank for almond species includes 138 genotypes of which 9 species: *Amygdalus bucharica, A. nana, A. intermedia, A. fenzliana, A. kotski, A. kuranika, A. scoparia, A. spinossa, A. webbii* (Branişte et al., 2006).

Currently, at RSFG Constanta there are 54 cultivars and selections (MADR).

The current assortment has the following autochthonous cultivars: Veronica, Mirela, Sandi, Cristi, Adela, April, Ana (created at RSFG Oradea) (Gavăt et al., 2013).

PERSPECTIVE OF INTRODUCTION OF NEW VARIETIES WITH LATE FLOWERING

At present, the growing demand on the market for nuts, places the almond culture in an interesting position in which the production of almonds with high quality organoleptic and nutritional produced will be needed to meet growing national and global demand (Socias et al., 2017). Although this culture is widely spread in the world, the production is limited by ecological factors such as late spring frost (Melhaoui et al., 2019).

Cold resistance decreases as the trees get closer to entering the vegetation (Opriță et al., 2022).

Breeding late flowering cultivars to avoid spring frosts is one of the most important objectives of the researchers in various European almond breeding programs (Rubio et al., 2017).

Like other temperate trees, the almond tree is at rest during the winter and is generally without leaves. In these conditions, it is strong in its ability to endure very low temperatures. Nevertheless, the almond is very sensitive to cold during flowering and early fruit development. During this period, depending on many factors, in particular phenological stage, temperature and exposure time, temperatures below -1°C or -2°C can cause crop loss (Guillamon et al., 2022).

Therefore, breeders are interested in delaying the flowering time of new cultivars so that they flower when the danger of frost is minimal (Dicenta et al., 2017).

The cultivars that bloom late and ripen early are considered as being very valuable from the biological and practical point of view, because the fruit buds differentiation for the next yield performs until late autumn; having a long dormant period these cultivars are more resistant to temperatures variation arising at the end of winters and ensure high yields (Gavăt et al., 2015).

The cultivars flowering time is therefore very important and largely determines the success of commercial exploitation (Socias et al., 2017).

In present, here are currently two major genetic improvement programs: Davis University Californian program (Dale E. Kester and R. Janes) and the European programs, in which Spain (A. Felipe and R. Socias), Italy (F. Monastra and C. Fideghelli) and France (Ch. Graselly).

In the former Soviet Union breeding begun in the 1930's at Nikita Botanical Garden (Yalta, Crimea) by A. Richter and A. Yadrov who focusing on tree hardiness and frost resistance, as well as late bloom and productivity. Many cultivars were released in this program such as "Yaltinskij", "Primorsrkji", "Myagkoskolupyj" (Richter, 1972).

In the 1960's breeding work begun at INRA Bordeaux, followed later at Montfavet, France, by Ch. Graselly and later by H. Duval in the 1990's, with the aim of obtaining late flowering. The French breeding program was the most popular program in Europe for many years, with the successful introduction of "Ferragnes" and "Ferraduel" in 1967, and the late-flowering, self-incompatible cultivar "Feralise" and "Ferrastar" in 1970s (Graselly and Raynaud, 1980). In 1994 and 1997, Lauranne and Steliette cultivars were introduced to the orchards (Godini et al., 2001).

Scion breeding activity was introduced in the 1970's, lasting 20 years, in Italy (F. Monastra at ISF Rome), in Greece (D.K. Stylianides and G. Syrigianidis at Naoussa) and in Tunisia (A. El Gharbi and B. Jraidi at INRAT Ariana).

The breeding program from CEBAS-CSIC (Murcia, Spain) has its main objectives based on late bloom time (Socias et al., 2010).

The first releases in the late 1990's were the "Antoaneta" and "Marta" (Egea et al., 2000). Two more recent cultivars, "Penta" and "Tardona" are characterized by their exceptionally late blooming time (Dicenta et al., 2009).

"Tardona appears to be the latest flowering cultivar ever released (Dicenta et al., 2010).

A large number of cultivar and germplasm evaluation took place in other countries, including: Romania, Bulgaria, Morocco, Iran, India (Socias et al., 2017).

Thus, from a breeding perspective, cultivars have to fulfil many requirements to be successful, even when they are intended for a single purpose. Almond quality is determinate largely by market acceptance (Socias et al., 2008).

The most effective way to prevent damage caused by low temperatures is to use late blooming cultivars, being the best method to avoid crop damage (Imani et al., 2011).

The introduction of late flowering cultivars can bring a special economic contribution to the almond culture by increasing the productivity and quality of the fruits (Neagu, 1975).

MODERN CULTIVATION TECHNOLOGIES

The almond tree is an economically important crop. Direct benefits get generated by the adoption of new technologies in the target area (Wani et al., 2021).

In order to constantly improve the production of almonds, it is also necessary to improve the increasingly efficient and sustainable cultivation techniques by choosing optimal planting distances and suitable canopy (Maldera et al., 2021).
The application of an advanced agricultural technique, of new, perfected technologies that allow doubling, tripling of crop the same surface is conditioned by the choice of suitable cultivars (Neagu, 1975).
Planting density, which refers to the number of trees per unit area, determines numerous aspects of orchard profitability including the precocity of production, yield in the mature stage of the orchard, the initial investment, the cost and type of orchard management and the commercial life of the orchard. An increase in planting density will lead to earlier production, thus reducing the non-productive stage of the orchard (Socias et al., 2017).
A new culture system in the super high densities (SHD) one which is known as a "sustainable and efficient system" due to the optimized use of natural resources such as soil, water and agronomic inputs such as fertilizers and chemical treatments (Maldera et al., 2021).
The first crop of SHD almonds was in Spain, in 2010, and soon after several almond producing countries such as Italy, Portugal started to adopt the super intensive system of culture (Maldera et al., 2021).
The productivity of modern high density planting orchards is a function of their light interception (Grapadelli and Lasko, 2007).
The growth habits of almond trees did not evolve with farming in mind. An almond tree left to grow freely would be difficult to harvest and manage on a commercial scale. The primary goal of both training and pruning is to exploit the natural tendencies of the tree to create and maintain a habit that will produce excellent yields and facilitate cultural practices. In the first years, the tree is trained to a structure that will support future crop weight and allow for cultural practices, while minimizing cuts which could decrease early yields (Socias et al., 2017).
The implantation of these novel training systems can give a significant impact (Casanova et al., 2019).
Once the tree structure has been established, pruning principally facilitates cultural practices such as spraying and harvesting and removes dead and diseased wood. Pruning has been seen as a way to invigorate tree growth (Socias et al., 2017).
New canopy was designed, by increasing the number of three axes to two in Bibaum, Bi-axis or to three axes in Parallel Trident (Stănică, 2019).
The innovative feature of SHD almond orchards is the possibility to fully mechanize the orchard management operations (Iglesias, 2019).
The pruning is done mechanically by reducing the height and width of the fruit trees row, creating a hedgerow (Cioacă and Stănică, 2021).
A perspective of the strengths of the modern SHD culture, we have in table 3, namely the cultivars, planting distances, training and pruning.

Table 3. Some characteristics of SHD system

Cultivars	Late to very late blooming	
Training and planting distances	Vertical Axis	4.0-4.5 x 1.5
	Parallel U	4.0-4.5 x 1.5-2.0
	Trident	4.0-4.5 x 2.5-3.0
Pruning	Mechanical	

Source: Stănică., 2019

CONCLUSIONS

The almond tree (*Amygdalus communis* L.) is a fruit species of great importance, which finds favorable growing conditions in certain areas in Romania and stands out due to the many properties of its fruit which have a long shelf life.
The popularity of almond growing demand resulting from this popularity make it necessary to provide cultivars with late flowering which correspond to the current climatic conditions and the adoption of modern cultivation technologies.
Almond cultivation offers interesting and economically sustainable prospects.

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APPLE TREES FERTILIZATION AND ITS INFLUENCE ON THE POTASSIUM CONTENT IN SOIL AND PLANTS IN CORRELATION WITH CALCIUM AND MAGNESIUM ABSORTION

Diana Elena BOLOHAN, Mariana VOLF, ElenaLiliana CHELARIU, Lucian RĂUS

University of Life Sciences “Ion Ionescu de la Brad” Iași,
3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: rauslucian@uaiasi.ro

Abstract

The paper presents how the soil fertilization with NPK in different doses and combined with two types of foliar fertilizers, applied to an apple tree orchard, changes the potassium content in soil and leaves. For a better understanding of the results, chemical analysis of the leaves and of the soil at two depths: 0-20cm and 20-40cm were chemically performed. The study was carried out in an apple orchard, located in the Didactic Farm “V. Adamachi" that belongs to the University of Life Sciences from Iasi (IULS), Romania. Results shows that fertilizers applied in the soil had a positive influence, increasing the soil content of potassium closer to the optimal range: 200-300 ppm. However, the content of potassium in the apple tree leaves stays low and below the optimum condition. The control (no fertilizers applied) had the lowest content for Kt (0.99%) in the leaves. Studying the content of calcium (mean – 1.65%) and magnesium (mean - 0.74%) in the dry matter of the leaves we see that there are not significant differences between the fertilization variants. Their values exceed the optimal limits.

Key words: *apple orchard, dry matter, foliar fertilizers, optimal content of potassium, NPK.*

INTRODUCTION

Potassium is a nutrient with major importance in the biochemical processes that take place in the plant: protein synthesis, lignin formation, transport and storage of carbohydrates imprinting resistance to drought, diseases and pests (Cheng, 2013; Malvi, 2011).

Potassium is the most abundant cation in the plant cell.

The harvests are directly dependent on the presence in the soil in optimal quantities but also in the plants, where it directly influences both the quantity and especially the quality of the production (Papp et al., 2004). The use of different potassium fertilizers and different doses in a Golden Delicious’ orchard did not determine the increase of the yield, but its quality (Szewczuk et al., 2008).

Its action in the soil and plant cannot be understood as a stand-alone one, but its evolution in both environments is directly related to nitrogen and phosphorus, with which it achieves high and stable productions.

Increasing the concentration of some ions can lead to the blocking of others or reduce their absorption by plants, thus the application of potassium fertilizers caused a decrease of foliar nitrogen (Holb et al., 2009).

In the soil, potassium is important especially through its accessible fraction to plants, which represents approximate 1% of the total soil potassium. Specific to this form is the fact that it can undergo, in specific soil conditions, by decreasing the content accessible to plant nutrition. Suboptimal concentrations of potassium in the soil will force the plants to absorb it against the electrochemical ingredient (Ragel et al., 2019). The harvests are at high levels, when potassium represents more than 5% of the amount of exchangeable bases (Davidescu & Davidescu, 1999).

The texture, the pH, the buffering capacity of the soil, as well as the alternation of dry and wet periods, determine the change of potassium into fixed forms and therefore inaccessible to plants. Soil acidification leads to a decrease in potassium in the leaves with negative effects on fruit quality (color, sugar content) (Raese, 1995) while Fazio et al. (2012) noted that the accessibility of calcium, phosphorus and molybdenum increases with increasing pH, while the absorption of zinc, magnesium and potassium is not influenced by pH. In the same

register, Malvi (2011) said that high pH soils negatively affected potassium absorption, which will aggravate the K/Mg antagonism.
This study was undertaken to improve apple nutrition with potassium on calcareous soil through minimal soil fertilization completed with foliar fertilizers with macro and microelements. The second aim was to increase the concentration of available potassium in the soil. During the three years of experiencing, observations were made on the evolution of the potassium content in the soil, the accumulation of potassium, calcium and magnesium in the plant.

MATERIALS AND METHODS

Location and experimetal design

The study was conducted for three years (2019-2021) in the Didactic Farm V. Adamachi of IULS, Iaşi County, in an apple tree orchard, Idared variety, grafted on MM-106 rootstock, spacing between trees is 4 x 4 m. Orchard soil type is aric cambic chernozem, rich in calcium. Prior setting the experiment, the activity of hydrogen ions from a soil sample is measured potentiometric, in aqueous suspension (1:2.5): the pH value is 8 for 0-20 cm and 8.3 for the 21-66 cm depth. For the same depths, through Scheibler method, was determined $CaCO_3$ (%) in soil: 4.8% (0-20 cm) - 7.8% (21-66 cm), and Ca^{2+} (13.3%-16%), Mg^{2+} (0.45%-0.62%) analyzed with atomic absorption spectrophotometry method
Fertilizing treatments were randomized within one block; groups of three trees; in three replications. In the experience were studied 9 variants of fertilization with mineral and foliar fertilizers, including the control: V1 - Control variant - no fertilizers; V2 - NPK 15.15.15 - 180kg ha^{-1} active ingredient, V3 - NPK 15:15:15 - 270kg ha^{-1} active ingredient, V4 - Pentakeep, V5 - Cropmax, V6 - V2+Pentakeep, V7 - V3+Pentakeep, V8 - V2+Cropmax, V9 - V3+Cropmax.
An NPK complex fertilizer, with 15:15:15 NPK ratio was used for soil fertilization, at a dosage of 180 kg ha^{-1} and 270 kg ha^{-1} active ingredient. Cropmax fertilizer was included in the experiment being a bio-stimulant compatible with organic farming, rich in nutritive elements, amino acids and vitamins, recommended for the efficiency of mineral fertilization. Cropmax has 0.2% N, 0.17% P, 0.017% K, 0.001% Ca, 0.033% Mg and microelements. Pentakeep Super fertilizer contain: 16% N, 2.18% P, 2.49% K, 0% Ca, 1.8% Mg, microelements and 5-aminolevulinic acid, with a much more concentrated composition in nutritious elements applied on trees foliage, recommended as well to complete mineral fertilization.
The mineral fertilization with NPK 15:15:15 was applied and incorporated in the topsoil, 1/3 in autumn and 2/3 in early spring. Foliar fertilizers (Pentakeep - 2 L/ha and Cropmax - 4 L/ha) were sprayed three times, received when the fruit was 5 mm in diameter, and every two weeks after. The spraying of the trees was carried out using the atomizer with fine spraying.

Sampling and Analysis

Annually, four soil samples were taken from each plot at the beginning of the vegetation period, before fertilization, from the upper 0-20 cm layer and 20-40 cm by using manual sampling equipment. The samples were brought to the laboratory, air-dried, grounded, passed through a 2 mm sieve, homogenized and stored in boxes until the chemical analyzes were performed.
Available potassium content in soil (K-AL) has been determined by treating the soil sample with ammonium lactate acetate (AL), pH - 3.7 (Egner-Riehm-Domingo method), precipitation of calcium from the solution with oxalic acid (10%). Potassium was quantified by the flame-photometry method.
Leaves were harvested from each tree were the soil samples were taken, in middle of July, from the middle part of the annual shoots. The plant samples were washed with distilled water, buffered with filter paper and dried at ambient temperature, after which they were ground and stored in glass jars.
Total potassium content in leaves (Kt) was determined by mineralizing the sample with sulfuric and hydrochloric acid followed by flame photometry dosage of the solution. Calcium and magnesium content in leaves was determined through atomic absorption spectrophotometry method (AAS). For AAS, plant samples were calcined until whitish ash

and solubilized with 5 ml of hydrochloric acid (25%).

Statistical analysis was performed over the results using analysis of variance (ANOVA). Significant differences between means were identified using the least significant difference (LSD) test.

RESULTS AND DISCUSSIONS

In order to maintain and sustain soil fertility in potassium it's recommended to follow other indicators, as: calcium and magnesium content and ratio between Ca, Mg and K. Buffer capacity of the soil is another indicator that influence the availability of the potassium in soil, knowing that there is a good correlation related to the soil properties and clay content (Shanker & Seth, 2018).

Soil fertilization and combined fertilization variants record increases in mobile potassium content for both depths. For the depth of 0-20 cm, the values are 214.1-239.8 ppm K-AL, values that fall within the state of optimal provision of the soil with potassium, compared to the depth of 20-40 cm where the values (128.5-172.3 ppm K-AL) show poor potassium supply for apple crops (Table 1)

As expected, the K-AL content for the exclusively foliar fertilization variants show insignificant differences compared to the control. The optimal values of the mobile potassium content in the soil for apple orchards are 200-300 ppm (Lăcătușu, 2016), and the K-AL in this study lies in the optimal range only in the 0-20 cm layer.

Table 1. Fertilization influence on potassium content (K-AL, ppm) in soil (three years mean)

Variants	K-AL ppm (0-20 cm)	Mean difference	K-AL ppm (20-40 cm)	Mean difference
V1 - Control	167.2	-	124.2	-
V2- 180 kg ha^{-1} NPK	214.1	46.9	157.9	33.7
V3 - 270 kg ha^{-1} NPK	223.6	62.4	164.5	40.3
V4 - Pentakeep	176.0	8.8	128.5	4.3
V5 - Cropmax	170.9	3.7	128.5	4.3
V6 - V2+Pentakeep	230.3	63.1	165.7	41.5
V7 - V3+Pentakeep	237.4	70.2	172.3	48.1
V8 - V2+Cropmax	227.8	60.6	158.2	34.0
V9 - V3+Cropmax	239.8	72.6	167.1	42.9
	*LSD 5%	41.16	*LSD 5%	24.00
	**LSD 1%	49.86	** LSD 1 %	28.41
	***LSD 0.1%	59.22	***LSD 0.1%	33.78

LSD - least significant difference

Foliar analysis for potassium, in apple leaves, highlighted a series of aspects related to the level of Kt % in plants, correlated with its content in the soil, strongly depended on the fertilization variants. Potassium content in apple leaves in July could be sorted into the low to medium potassium supply for all fertilization variants. The exclusively foliar fertilized variants raise leaves content in potassium to 1.01% Kt when using Cropmax and 1.11% Kt when using Pentakeep, statistically insignificant increases in potassium content.

During the period of vegetation, the nutrients content varies in apple leaves (Mengel & Kirkby, 2001). The analyses should be performed in early stage in order to determine tree nutrition disorders. It is difficult to adjust its deficiency or excess in the late growing cycle (Uçgun & Gezgin, 2017).

The researches carried out on the dynamics of potassium absorption and its content in apple leaves, shows that potassium concentration in leaves, in July, states in the low range (Papp et al., 2004), even the K-AL content in soil has increased over the experimental years. Similar values have been noted in an organic apple orchard in Hungary (Nagy & Holb, 2006).

The variant with the highest total potassium content is V7, with 1.35% Kt, which means an optimal supply, with a statistically very significant difference compared to the control. Thus, the total potassium (Kt) in plants, recorded for this fertilization option, correlates with the mobile potassium (K-AL) content in the soil. It results that root fertilization in maximum NPK doses combined with foliar Pentakeep determines a normal active absorption of potassium (Table 2).

Table 2. Influence of differentiated fertilization on total potassium content in apple leaves (Kt, %)

Variants	Kt (%)	Percentage increase	Mean difference	Coefficient of Variation, %
V1 - Control	0.99	100	-	6.20
V2	1.19	120.2	0.20	13.40
V3	1.26	127.3	0.27	12.52
V4	1.11	109.4	0.12	9.56
V5	1.01	104.4	0.02	3.59
V6	1.19	120.2	0.20	11.04
V7	1.35	136.7	0.36	17.27
V8	1.17	118.5	0.18	20.18
V9	1.26	127.3	0.27	12.34

*LSD 5% - 0.20; **LSD 1% - 0.27; ***LSD 0.1% - 0.32

Increasing the concentration of potassium in soil leads to optimal accumulation of it in the plant (Kuzin & Solovchenko, 2021). Among the potassium content in the soil, at 20-40 cm depth, and the total potassium determined in the apple leaves, a positive relationship was identified; 88.04% of the variation in total potassium content in plant can be attributed to the variation of the accessible potassium in soil (Figure 1).

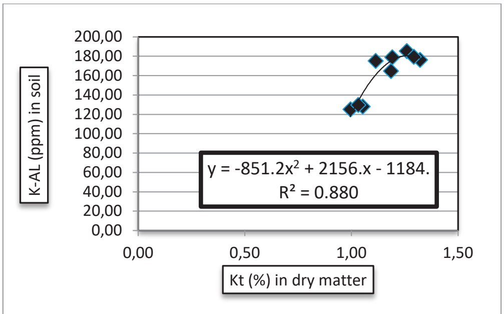

Figure 1. Dependence relationship between the available potassium in soil and total potassium in leaves

Calcium imprints the firmness of the fruit pulp, neutralizes organic acids, stimulates the formation of absorbent bristles on the root, favors the processes of fruit formation and ripening (Davidescu & Davidescu, 1999).
Regarding the calcium content in the apple leaves, it records values between 1.55 - 1.75%, that express an excessive state of insurance with this element (Lăcătuşu, 2016) (Table 3).
The statistical analysis shows that increases/decreases of this element in plant tissue are not significant and not in direct relation with the fertilization options. The concentration of this element is determined by the high content of calcium carbonate in the soil (7.8% $CaCO_3$).

Table 3. The influence of fertilization on calcium accumulation (Ca, %) in leaves (three years mean)

Variants	Ca (%) Mean ± SD	Mean difference	Coefficient of Variation, %
V1 - Control	1.62 ± 0.16	0	9.62
V2	1.60 ±0.09	-0.021	5.70
V3	1.65 ±0.17	0.030	10.23
V4	1.65 ± 0.09	0.030	5.71
V5	1.55 ±0.33	-0.072	21.33
V6	1.58 ± 0.21	-0.041	13.30
V7	1.68 ± 0.21	0.060	12.59
V8	1.75 ± 0.14	0.130	8.13
V9	1.74 ± 0.13	0.120	7.55

*LSD 5% - 0.127;**LSD 1% - 0.132;***LSD 0.1% - 0.138

The analyses determined that calcium content in dry matter is optimal in this study, and for some variants of fertilization Ca recorded a high state of insurance of the trees. In an experiment Dilmaghani et al. (2004) noticed a range of potassium and calcium content in leaves that exceeded their critical limit values, even so the calcium concentration was low in fruits, due to its low mobility.
Magnesium has a major role in photosynthesis and is contained in tissues at values denoting a high state of supply, 0.70-0.83% (Davidescu & Davidescu, 1999). Although there are many studies on magnesium deficiency of apple trees, there is no information on the effect of high magnesium content.
Through fertilization with Pentakeep, the apple trees benefited of an intake of magnesium. Very significant increases were recorded for the fertilization variants V7 and for the exclusive fertilization with Pentakeep. For the rest of the fertilization options, the increases were insignificant (Table 4).
It is known that a high content of potassium in the soil can cause a decrease in the absorption of magnesium, resulting in a decrease in the

concentration of Mg in leaves. It has not been proven, in all cases, that a high concentration of this element in the soil can cause a decrease in potassium intake.

Table 4. The influence of fertilization on magnesium accumulation (Mg, %) in leaves (three years mean)

Variants	Mg (%) Mean ± SD	Mean difference	Coefficient of Variation, %
V1 - Control	0.70 ± 0.05	-	7.03
V2	0.72 ± 0.07	0.02	9.57
V3	0.77 ± 0.05	0.07	5.93
V4	0.83 ± 0.03	0.13	3.43
V5	0.70 ± 0.05	0	6.79
V6	0.75 ± 0.08	0.05	10.13
V7	0.82 ± 0.04	0.12	5.32
V8	0.72 ± 0.07	0.02	10.30
V9	0.66 ± 0.07	-0.04	10.41

*LSD 5% - 0.09;**LSD 1% - 0.10; ***LSD 0.1% - 0.12

K/Ca, K/Mg and Ca/Mg are indicators that refers to antagonism relationships between these cations and are a better expression of the nutrition state of the plants. In this study the K/Ca ratio has values between 0.61-0.80, K/Mg ratio has values between 1.34-1.91 and Ca/Mg ratio has values between 1.99-2.64 (Table 5). These ratios have under - optimal values compared to other studies, where K/Ca = 0.87, K/Mg = 3.9-6.0 and Ca/Mg = 4.55 (Füleky, 1999) due to the low content of potassium in leaves. It is recommended to use foliar fertilization in order to achieve optimal calcium in fruits (Mengel, 2002).

Table 5. Ratios of potassium, calcium and magnesium in apple leaves (three years mean)

Variants	K/Ca	K/Mg	Ca/Mg
V1 - Control	0.61	1.41	2.31
V2	0.74	1.65	2.22
V3	0.76	1.64	2.14
V4	0.67	1.34	1.99
V5	0.65	1.44	2.21
V6	0.75	1.59	2.11
V7	0.80	1.63	2.02
V8	0.67	1.63	2.43
V9	0.72	1.91	2.64

CONCLUSIONS

Soil fertilization causes significant increases in the potassium content of the soil.

The concentration of potassium in the plant recorded increases for all fertilization options, there is a positive correlation between the increase of the accessible potassium content in the soil and the increase in its content in the leaves.

The accumulation of calcium in plants is not influenced by the application of fertilizers, but is attributed to the high concentration of $CaCO_3$ in the soil. Its values denote good insurance with this element.

Fertilization with Pentakeep causes significant increases of magnesium content in apple leaves. All experimental variants have a high magnesium content that lies in the high state of insurance for apple trees. All three cations play a major role in obtaining high quality fruit.

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PEACH AND NECTARINE FRUIT CHARACTERIZATION FOR SEVERAL NEW CULTIVARS GROWN IN THE BUCHAREST AREA

Alexandru BUCUR[1], Ana Cornelia BUTCARU[2], Cosmin Alexandru MIHAI[1], Florin STĂNICĂ[1, 2]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Centre for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

Fruit physicochemical parameters are essential for evaluating the new cultivars when tested in new planting conditions. The article presents the fruit's biochemical characteristics during four-year research (2019-2022) for 30 peach and nectarine cultivars. The orchard was established in 2017 in the Experimental Field of the Faculty of Horticulture in Bucharest with Romanian and foreign cultivars grafted on Myrobalan 29C, Saint Julien A, Adesoto, and GF677 rootstocks. Vertical Axis and Trident were used as planting systems, and an integrated orchard technology was applied. The size, average weight, flesh firmness, soluble solids, dry matter, titratable acidity, fructose and glucose content, and absorbance index were measured/determined for fruit evaluation. The results present the range intervals for all monitored parameters and the distribution of the cultivars on clusters depending on the physical and biochemical parameters. At the same time, the rootstock and system planting influence on the fruit parameters are highlighted.

Key words*: total soluble solids, dry matter, total acidity, DA-meter.*

INTRODUCTION

The Rosaceae family includes the peach (*Prunus persica* (L.) Batsch) and the nectarine (*Prunus persica* var. *nectarine* Maxim). Due to the fruit's simple adaptability to many ecological situations, early fruit set, and lengthy harvest time, peaches are widely planted. Low winter temperatures and late spring frost at higher elevations limit peaches and nectarines (Kuden et al., 2018). According to FAO data, in 2021, the top-producing nations of peaches and nectarines worldwide were China (8,850,345 tons), Italy (1,466,753 tons), Spain (1,221,698 tons), the United States (1,137,075 tons), Greece (795,851 tons), and Turkiye (555,825 tons). Peach and nectarine production holds a significant global position with a planted area of roughly 1.5 million hectares. Fruit quality in peaches and nectarines is primarily influenced by genotype. Other elements such as rootstock, the location of the fruit in the canopy, pruning and thinning techniques, and the yearly climate are also known to have an impact (Fonti i Forcada et al., 2013). Peaches and nectarines' suitability for consumption primarily depends on their sweetness, and it has been shown that there is a positive correlation between the sugar and malic acid content of the fruits and their flavor (Orazem et al., 2011). Large-fruited nectarines became available in the 1980s, and it was expected that nectarine cultivars would eventually take over the market for fresh *P. persica* in the 21st century (Hough, 1985). Nectarines have increased in popularity over the past 20 years and currently makeup roughly 30% of all peach and nectarine types (Byrne, 2002). Consumer preferences vary depending on consumption patterns, but customers prefer fruits high in sugar yet low in acidity (Rossato et al., 2009). The sugar profile refers to the proportion of each type of sugar in a particular fruit, being quite different from the total sugar content, that is, the sum of the four most significant sugars in fruit (sucrose, glucose, fructose, and sorbitol).

The primary sugar in peach fruit is sucrose (Robertson et al., 1990). This disaccharide is crucial as a fruit flavor antioxidant, sweetener, and energy source (Huberlant & Anderson, 2003). There are also lesser amounts of other

sugars, such as glucose, fructose, and sorbitol (Moriguchi et al., 1990).
Since it is sweeter than sucrose and glucose, fructose is a significant monosaccharide in fruit flavor (Pangborn et al., 1963).
Furthermore, because it encourages the growth of bifidobacteria and lactobacilli in the gastrointestinal tract, fructose has been shown to have positive benefits on digestive health (Muir JG et al., 2009).
The present study aims to highlight the qualitative indices and biochemical attributes of 14 peach cultivars and 16 nectarine cultivars to spread the knowledge of this worldwide consumed fruit with essential functions for human health.

MATERIALS AND METHODS

The study was carried out in the experimental field of the Faculty of Horticulture in Bucharest. Both peach and nectarine cultivars were led on two different planting systems: Vertical Axis with 4.0 x 1.5 m (1,666 trees/ha - 1,666 axis/ha) and Trident with 4.0 x 2.0 m (1,250 trees/ha - 3,750 axis/ha). Integrated management, including fertilization, pest and disease management practices, and irrigation, were used to grow the trees. Tree branches were thinned to the same relative fruit: foliage ratio, simulating commercial culture.
The rootstocks were ’GF677’, ’St Julien A’, ’Mirobolan29C’, and ’Adesoto’.
Fruit quality is a broad notion that includes sensory qualities (such as appearance, texture, flavor, and aroma), nutritional value, mechanical qualities, safety, and defects (Crisosto & Costa, 2008). Together, these attributes give the fruit a degree of excellence and an economic value (Abbot, 1999).
To determine the fruit quality parameters, a collection of 30 cultivars (peach and nectarine) was evaluated between 2019 and 2022 for caliber and weight, TSS (total soluble solid), DM (dry matter), titratable acidity (TA), fructose and glucose content, absorbance index (IAD), and fruit flesh firmness.
For each cultivar, 2 or 3 trees were used, and ten fruits per cultivar were sampled in analysis. All fruits were harvested at the commercial ripe stage, when fruits softened, had a yellow or orange ground color (which was also characteristic of each cultivar), and were simple to separate from the tree. To guarantee consistency in maturity grade, one individual only harvested them. Maturity dates ranged from mid-June to mid-September, depending on genotype. The yield (kg per tree) and total number of fruits were recorded for each cultivar. The average weight was estimated using these measurements in 20 fruits in a representative sample (Crisosto et al., 2001). The juice TSS was measured with Krüss DR301-95 refractometer, and data were given as °Brix.
The titratable acidity (TA) was measured in the fresh fruit juice. The fresh juice was measured with a pH electrode and diluted with distilled water for titration to an end pH of 8.1 with 0.1 mol L^{-1} NaOH according to the AOAC method (AOAC, 2001). Data were given as g malic acid per 100 g fresh weight (FW) since this was the dominant organic acid in peach (Wills et al., 1983).
Flesh firmness was determined on opposite sides of the equator of each fruit with a penetrometer Turoni with an 8 mm diameter probe on ten fruits from each tree. Data averages were given in kgf cm^{-2} (Harker et al., 2002).
Dry matter content in a sample was determined by weighing it before and after being incubated at 105°C in a forced air draft oven for 24 hours. The result was expressed as g dry weight (DW) g^{-1} fresh weight (FW) (Di Vaio et al., 2015).
For the descriptive statistics of the data, Microsoft Excel 2016 and IBM SPSS v. 28.0.1.1 with a significance level of $p = 0.05$ were used.

RESULTS AND DISCUSSIONS

At the time of commercial harvest, high variations existed in the values of fruit diameter (Table 1), in peach and nectarine, being influenced by both genotype and canopy shape; the values obtained varied between 50-80 mm.
In nectarine, the Vertical Axis canopy imprinted higher fruit weight values in most cultivars, ranging between 64 g (Early Sun Grand/SJA) and 150 g (Honey Royal/GF677). Fruit diameters were between 55.5-68.73 mm.
Some cultivars, such as Caldessi2000/SJA_A, Nectabelle/GF677_A, and Nectagrand4/SJA_A, exhibited lower fruit diameter. These cultivars tend to produce smaller and lighter fruits, which might be preferred by consumers seeking

nectarines with smaller sizes and delicate flavor profiles. Most of the studied nectarine cultivars exhibited moderate diameter values ranging from 54 to 64, representing a balanced fruit size suitable for a wide range of consumer preferences (Table 1).

At the peach cultivars, the Trident canopy shape tended to lead to bigger fruits. Some cultivars, including Royal Summer/GF677_A, Sweet Dream/GF677_A, and Sweet Juana/GF677_A, displayed diameter values ranging between 60 and 64, being into the category of medium-sized fruits, catering to consumers seeking a balanced size and taste experience (Table 1).

Several cultivars, such as Royal Summer/GF677_T and Cardinal/M29C_T, displayed high values exceeding 65, known for producing large and heavy fruits and being visually appealing.

Royal Summer/GF677 cultivar had bigger fruits than Royal Summer/Saint Julien A on both canopy shapes. It produced more than 70% of fruit belonging to the AA category (74-81 mm), conforming to the retail group and supermarket chain standards of European markets (Kader & Mitchell, 1989).

The TSS level of peaches and nectarines showed significant differences among cultivars ranging from 8.00 ± 16.00°Brix (Table 1). All these TSS values were over 8°Brix, considered the minimum TSS established by the European Union to market peaches and nectarines (Commission Regulation 1861/2004). For high-acid cultivars, consumer adoption in American markets was highest when TSS > 10% to 1. In contrast, for low-acid cultivars, the degree of acceptance was at TSS 15% to 16%, above 90% (Crisosto & Crisosto, 2005). Fruit biochemical concentrations at maturity result from changes produced throughout fruit growth. Previous analyses of biochemical compound developmental alterations have mainly concentrated on commercial cultivars. According to (Chapman & Horvat, 1990) and (Chapman et al., 1991), physiological maturity for peaches was characterized by the highest sucrose and lowest quinic acid contents.

The results show a variation in the range of °Brix values among the studied peach and nectarine cultivars.

At nectarines, the Trident canopy registered higher values than the Vertical Axis. Cultivars with the highest TSA values were Nectareine/M29C (14.293°Brix), Honey Late/SJA (13.44°Brix), and Big Top/GF677 (13.75°Brix). The cultivars Big Bang/GF677 (8.733°Brix) and Big Fire/GF677 (9.623°Brix) were the lowest values.

At peaches, most of the cultivars presented similar TSS values on both planting systems, the highest being at Sweet Henry/Adesoto and Sweet Juana/GF677, known for their exceptional sweetness, making them highly desirable for those who prefer intensely sweet flavors.

The lowest values were Sugar Time/Adesoto and Royal Glory/Adesoto, which may have a milder sweetness, appealing to individuals who prefer fruits with a less pronounced sweetness.

Royal Summer on GF677 rootstock registered higher values for TSS than on the SJA.

According to (Moing et al., 2003), peach fruits are typically regarded as inedible when there is a high acid and extremely low sugar content. Changes in sugar and acid concentrations during fruit maturation in *P. persica* have been widely studied (Sandhu et al., 1983; Selli & Sansavini, 1995). In lower levels, sorbitol, a sugar alcohol, is the next most abundant sugar in peach fruits after sucrose, glucose, and fructose (Moriguchi et al., 1990; Robertson et al., 1990). Malic, citric, and quinic acids make up the majority of the acids found in peach fruits (Sweeney et al., 1970; Wills et al., 1983); lesser amounts of shikimic acid have also been found (Wu et al., 2002). Since these sugars and acids were found to have a significant impact on fruit flavor (Sweeney et al., 1970; Jensen, 1985; Esti et al., 1997), we searched to identify patterns between cultivars and system plantings that were linked to the quality of the final fruit.

Typically, the concentration of malic acid increased and then decreased as the fruit developed (Ishida et al., 1971; Liverani & Cangini, 1991). With fruit growth, the concentration of quinoic acid rapidly dropped. According to Chapman & Horvat (1990), quinic acid was the primary acid in young fruits, but it reduced as the fruit grew. Shikimic acid was present in peaches in small amounts (Wills et al., 1983), and (Wu et al., 2002) found that as the fruit matured, the concentration dropped.

Table 1. Caliber, firmness, and total soluble solids (°Brix) at nectarines and peaches between 2019-2022

Genotype/ Rootstock	Fruit weight (g)		Diameter (mm)		Firmness (kgfcm^{-2})		Total soluble solids (°Brix)	
	VA	T	VA	T	VA	T	VA	T
Nectarines								
Early Sun Grand/SJA	64.327 f	55.546 gn	55.546 gn	51.766 ef	2.698 bcdef	1.872 efg	9.077 d	10.518 bcd
Caldessi2000/SJA	70.085 ef	57.562 g	57.562 g	54.043 de	1.848 g	2.428 cde	7.590 e	10.827 bcd
Nectagrand1/SJA	73.283 ef	54.794 n	54.794 n	52.312 ef	2.001 fg	2.805 cd	9.160 d	10.510 bcd
Nectagrand4/SJA	74.463 ef	49.906 i	49.906 i	51.808 ef	2.139 efg	1.241 f	10.558 bc	10.573 bcd
Guerriera/SJA	81.059 de	63.251 bc	63.251 bc	55.857 d	3.383 ab	2.936 bcd	9.812 cd	10.793 bcd
Nectabelle/GF677	81.227 de	50.204 i	50.204 i	49.792 f	2.023 fg	2.459 cde	11.930 a	10.021 bcd
Honey Late/SJA	91.565 d	58.686 ef	58.686 ef	59.907 c	3.486 a	3.318 abc	9.830 cd	13.440 ab
Nectaross/SJA	95.167 cd	61.611 cd	61.611 cd	59.846 c	3.335 abc	2.784 cd	10.587 bc	11.450 abcd
Nectareine/M29C	107.477 bc	62.679 bc	62.679 bc	62.490 bc	3.319 abc	2.567 cde	11.987 a	14.293 a
Stark Red Gold/SJA	110.123 b	64.146 b	64.146 b	61.678 bc	2.449 defg	3.761 a	10.587 bc	11.535 abcd
Big Bang/GF677	115.620 b	53.609 n	53.609 n	52.293 ef	2.958 abcd	1.761 fg	10.035 cd	8.733 d
Big Fire/GF677	116.548 b	55.672 gn	55.672 gn	55.929 d	2.598 cdef	1.954 efg	9.560 cd	9.623 cd
Big Top/GF677	140.807 a	57.727 fg	57.727 fg	56.535 d	2.818 abcde	3.611 ab	12.438 a	13.175 abc
Maria Anna/SJA	145.954 a	63.470 bc	63.470 bc	62.786 b	2.830 abcde	2.245 cde	12.840 a	11.000 abcd
Honey Royal/GF677	150.840 a	68.731 a	68.731 a	67.570 a	3.072 abcd	3.321 abc	11.687 ab	12.030 abcd
Peaches								
Cardinal/M 29C	58.590 g	73.892 n	50.683 e	66.162 b	1.548 fg	2.808 bcd	11.470 bc	9.830 ef
Royal Summer/SJA	119.800 cbe	109.150 f	51.080 e	59.962 bc	2.436 cde	2.856 bc	8.550 c	10.010 ef
Sugar Time/Adesoto	87.360 f	69.606 n	54.361 d	55.012 cd	1.953 defg	1.896 ef	8.985 c	9.215 f
Red Top/M29C	122.100 cbe	97.408 g	54.501 d	60.196 cb	1.478 g	2.409 bcde	10.445 c	10.113 ef
Royal Glory/Adesoto	135.117 bce	112.817 ef	58.923 c	61.253 bc	1.896 efg	2.346 bcde	8.393 c	9.313 f
Sweet Henry/Adesoto	82.020 f	114.400 ef	59.822 c	64.700 b	2.180 cdefg	1.948 deff	13.880 bc	15.500 a
Sweet Juana/GF677	114.808 be	115.127 ef	60.794 bc	61.906 bc	2.721 abcd	2.944 bc	16.258 a	13.060 b
Royal Jim/Adesoto	109.865 e	117.474 ef	60.837 bc	62.088 bc	2.601 bcde	2.376 bcde	11.583 bc	11.248 d
Lucius/GF677	116.694 be	122.668 de	61.196 bc	62.088 bc	3.416 a	4.055 a	11.878 bc	13.123 b
Royal Majestic/Adesoto	124.907 cbe	88.440 g	61.924 bc	55.965 cd	2.723 abcd	3.230 b	10.037 c	9.538 ef
Sweet Ivan/GF677	138.978 bc	136.640 bc	63.106 b	62.601 bc	2.690 abcd	2.618 bcde	12.000 bc	11.583 cb
Sweet Dream/GF677	146.375 ab	130.193 cd	63.430 b	65.995 b	2.796 abc	2.301 cde	10.535 c	10.671 de
Royal Summer/GF677	115.190 be	232.212 a	63.927 b	74.070 a	3.253 ab	3.100 bc	16.630 a	11.537 cb
Nabby/GF677	117.380 be	139.114 bc	66.751 a	62.601 bc	2.674 abcde	2.641 bcde	9.193 c	9.660 ef
Gladys/GF677	163.443 a	144.870 b	66.842 a	64.788 b	2.278 cdef	2.924 bc	12.434 bc	12.365 bc

*VA- Vertical Axis; T - Trident

Table 2. Dry matter, acidity, the ratio between acidity and total soluble content, and absorbance index at nectarines and peaches between 2019-2022

Genotype/ Rootstock	Dry matter (%)		Acidity (mg malic acid/100 g fw)		Acidity/TSS		Total IAD	
	VA	T	VA	T	VA	T	VA	T
Nectarines								
Early Sun Grand/SJA	10.556 ab	11.667 abcdef	0.748 a	0.637 c	0.065 bc	0.066 abc	0.213 de	0.204 f
Caldessi2000/SJA	12.667 a	10.556 def	0.628 abc	0.529 cd	0.083 a	0.053 bcd	0.169 e	0.331 de
Nectagrand1/SJA	10.250 ab	10.222 ef	0.585 bc	0.532 cd	0.070 abc	0.043 cde	0.277 de	0.295 def
Nectagrand4/SJA	11.091 ab	10.889 def	0.644 abc	0.680 bc	0.063 bc	0.055 bcd	0.267 de	0.217 ef
Guerriera/SJA	9.778 b	14.083 a	0.633 abc	0.683 bc	0.064 bc	0.066 abc	0.309 cd	0.328 de
Nectabelle/GF677	12.667 a	11.125 cdef	0.266 ef	0.304 c	0.024 e	0.032 de	0.265 de	0.260 ef
Honey Late/SJA	11.900 ab	14.000 ab	0.381 def	0.333 ec	0.038 de	0.020 e	0.563 b	
Nectaross/SJA	10.625 ab	12.167 abcde	0.779 a	0.908 a	0.078 ab	0.080 a	0.257 de	0.871 a
Nectareine/M29C	10.778 ab	12.636 abcde	0.491 cd	0.512 cde	0.038 de	0.034 de	0.388 c	0.510 c
Stark Red Gold/SJA	12.000 ab	13.000 abcd	0.671 ab	0.875 ab	0.064 bc	0.077 ab	0.737 a	
Big Bang/GF677	9.400 b	9.400 f	0.374 def	0.599 c	0.042 d	0.069 ab	0.312 cd	0.285 def
Big Fire/GF677	10.222 ab	11.375 cdef	0.638 abc	0.665 bc	0.068 abc	0.071 ab	0.187 e	0.189 f
Big Top/GF677	11.083 ab	12.500 abcde	0.429 de	0.501 cde	0.037 de	0.043 cde	0.272 de	0.319 de
Maria Anna/SJA	11.800 ab	11.333 cdef	0.725 ab	0.729 abc	0.058 c	0.065 abc	0.214 de	0.231 ef
Honey Royal/GF677	11.909 ab	13.700 abc	0.285 ef	0.618 c	0.026 de	0.056 abcd	0.388 c	0.374 d
Peaches								
Cardinal/M 29C	11.333 bce	10.571 cdef	2.473 a	0.669 a		0.063 bc		0.387 bc
Royal Summer/SJA	11.833 a	10.667 cde	0.477 b	0.237 c	0.056 c	0.022 b	0.383 dce	0.291 cd
Sugar Time/Adesoto	9.833 cd	9.286 ef	1.188 b	0.276 c	0.023 c	0.034 bc	0.230 ce	0.365 bcd
Red Top/M29C	7.333 d	10.756 bcde	0.398 b	0.359 bc	0.266 a	0.041 bc	0.200 e	0.296 cd
Royal Glory/Adesoto	9.889 cd	10.126 def	0.266 b	0.225 c	0.080 bc	0.036 bc	0.602 ab	0.415 bc
Sweet Henry/Adesoto	14.273 b	13.017 abcd	1.013 b	0.670 a	0.062 c	0.043 bc	0.439 bc	0.300 cd
Sweet Juana/GF677	14.000 bc	13.657 ab	0.718 b	0.315 bc	0.031 c	0.018 b	0.419 bcd	0.605 a
Royal Jim/Adesoto	11.083 bcd	11.487 abcde	1.040 b	0.501 ab	0.211 ab	0.116 ab	0.674 a	0.597 a
Lucius/GF677	14.000 bc	14.253 a	0.919 b	0.156 c	0.053 c	0.012 b	0.468 bc	0.597 a
Royal Majestic/Adesoto	10.556 bcd	9.333 ef	1.111 b	0.618 a	0.108 bc	0.061 bc	0.420 bcd	0.453 abc
Sweet Ivan/GF677	11.769 bc	13.749 a	0.418 b	0.152 c	0.028 c	0.016 b	0.466 bc	0.514 ab
Sweet Dream/GF677	11.667 bc	9.314 ef	0.245 b	0.343 bc	0.146 abc	0.170 a	0.462 bc	0.315 cd
Royal Summer/GF677	11.833 bc	13.167 abc	0.357 b	0.288 bc	0.029 c	0.191 a	0.416 bcd	0.329 cd
Nabby/GF677	10.667 bcd	10.398 cdef	0.950 b	0.378 bc	0.028 c	0.038 bc	0.410 bcd	0.476 abc
Gladys/GF677	14.000 bc	13.003 abcd	0.714 b	0.604 a	0.138 abc	0.040 bc	0.548 abc	0.401 bc

*VA- Vertical Axis; T - Trident

The significant differences in several sugar traits observed between the cultivars confirmed the effect of sugar composition on the sensory quality of peach fruit (Colaric et al., 2005).

Conversely, their high acidity often favored yellow-fleshed peach and nectarine cultivars in Europe and America. As shown, sugar and acidity varied considerably for both white and yellow-fleshed peaches and nectarines (Day et al., 1997)

In the history of peach and nectarine breeding, fruit total acidity was a quality parameter that suffered changes in the newest cultivars.

In our collection, low acidity nectarines were Nectabelle/GF677, Honey Late/SJA, and Big Bang/GF677, and peaches were Royal Summer/SJA, Red Top/M29C, Royal Glory/Adesoto, Sweet Ivan/GF677, Sweet Dream/GF677, and Royal Summer/GF677.

The dry matter content in nectarines and peaches, Trident, and Vertical Axis had similar values to more cultivars (Table 2). At nectarines, varied between 9.4-14.083%, these values provide insights into the characteristics and properties of these cultivars.

Cultivars registered the highest values.

Guerriera/SJA_T and Honey Late/SJA_T, and the lowest by Big Bang/GF677 and Nectagrand1/SJA.

At peaches, the highest values were at Sweet Henry/Adesoto_A, Sweet Juana/GF677_A, Lucius/GF677_A, and Gladys/GF677_A.

Absorbance index (IAD) presented the maturity level according to this parameter that was analyzed in the fruits, most of the values being under 0.4.

The 14 peach and 16 nectarine cultivars (2019-2022) were grouped after the fruit traits using hierarchical clusters (Wanpeng et al., 2017; da Silva Torres et al., 2006).

At nectarines, five groups were formed for the basic parameters (a) Big Fire/GF677_T, Caldessi 2000/SJA_t, Nectagrand1/SJA_T, Maria Anna/SJA_T, Nectareine/M29C_A, Nectarine/M29C_t, Stark Red Gold/SJA_T, Guerriera/SJA_T, and Honey Royal/GF677_T; (b) Nectaross/SJA_A, Stark Red Gold/SJA_A, Honey Late/SJA_T, Nectagrand4/SJA_T, Big Top_GF677_A, Honey Late/SJA_A, Big Bang/GF677_A, Nectagrand4/SJA_A, Nectabelle/GF677_T; (c) Big Top/GF677_T, Nectaross/SJA_T, Big Bang/GF677_T; (d) Big Fire/GF677_A, Early Sun Grand/SJA_A, Caldessi 2000/SJA_A, Nectabelle/SJA_A, and Nectagrand1/SJA_A; (e) Maria Anna/SJA_A, Honey Royal/GF677/A, Early Sun Grand/SJA_T, and Guerriera/SJA_A (Figure 1). Nectareine/M29C and Nectagrand4/SJA presented a similar profile in both planting systems, while the others differed.

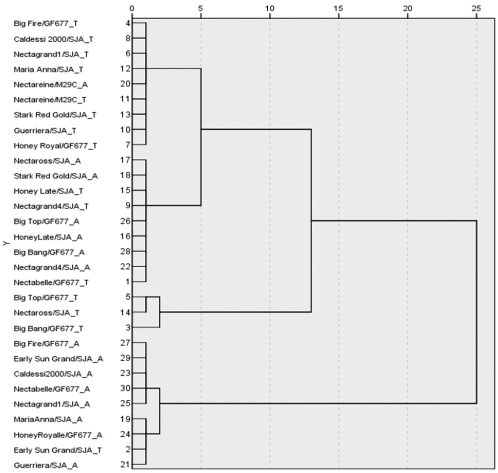

Figure 1. Nectarine cultivars grouped by fruit quality parameters

At peach, four groups were presented (a) Sweet Dream/GF677_A, Royal Glory/Adesoto_A, Royal Jim/Adesoto_T, Sweet Juana/GF677_T, Lucius/GF677_A, Red Top/M29C, Royal Majestic/Adesoto_T, Royal Jim/Adesoto_A, Royal Glory/Adesoto_T, Sweet Ivan/GF677_T, Sweet Juana/GF677_A, and Royal Summer/SJA_A; (b) Royal Summer/SJA_T, Sweet Ivan/GF677_A, Gladys/GF677_A, and Nabby/GF677_A; (c) Red Top/M29C_T, and Springbelle/M29C; (d) Sweet Henry/Adesoto_T, Sweet Henry/Adesoto_A, Gladys/GF677_T, Lucius/GF677_T, Cardinal/M29C_T, Nabby/GF677_T, Sweet dream/GF677_T, and Sugar Time/Adesoto_A; (e) Royal Majestic/Adesoto_A.

Royal Jim/Adesoto, Royal Glory/Adesoto, Sweet Juana/GF677, and Sweet Henry/Adesoto presented the same profile in both planting systems (Figure 2).

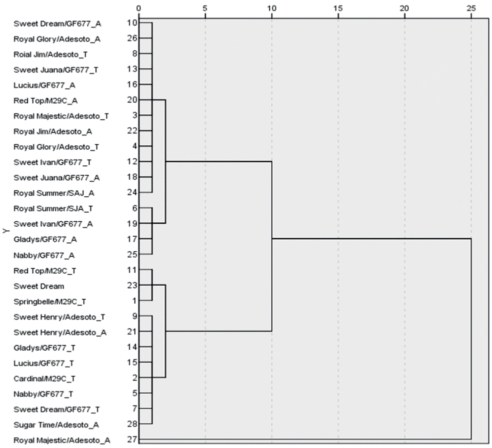

Figure 2. Peach cultivars grouped by fruit quality parameters

CONCLUSIONS

The caliber values from moderate-sized fruits suitable for various uses to larger-sized fruits with visually appealing characteristics, growers and consumers have a wide range of options to select from based on their preferences. The values were between 50-80 mm. Royal Summer/GF677 has bigger fruits than Royal Summer/Saint Julien A on both canopy shapes. Trident has bigger fruits than Vertical Axis at peach.

For total soluble solids (°Brix), the values ranged between 8-15°Brix, highlighting a wide range of sweetness levels when cultivars exhibiting exceptional sweetness are highly appreciated. Cultivars with moderate sugar content and suitable performance could balance flavor and tree management. Further research can explore additional sensory attributes and evaluate the performance of different canopy shapes to optimize fruit production and enhance consumer satisfaction.

Dry matter content varied between 7-15%. The titratable acidity content recorded higher values on the Trident canopy in some cultivars. It is important to note that taste preferences can vary among individuals, and some may prefer fruits with higher or lower acidity levels based on personal preferences. Other factors, such as sweetness, aroma, texture, and overall fruit quality, also contribute to the appreciation of a cultivar.

When evaluating the characteristics and properties of these cultivars, it is crucial to consider a combination of factors, including titratable acidity, dry matter concentration, fruit size, and adaptability to specific growing conditions, to determine their overall desirability and suitability for different uses.

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METHODS OF EVALUATING THE CHILLING AND HEAT REQUIREMENTS OF APPLE AND PEAR TREES

Ana Cornelia BUTCARU, Ruxandra GOGOȚ, Cosmin Alexandru MIHAI, Adrian ASĂNICĂ, Florin STĂNICĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: mcosminalexandru@yahoo.com

Abstract

The change in the occurrence of phenophases in fruit trees gave a challenge to climate change. Changes in average temperatures and rainfall and increased short- and long-term extreme events are already affecting crop yields worldwide. To avoid possible losses, it is necessary to provide helpful information to farmers in real time regarding initiating a particular stage of fruit tree development. Modern breeding programs have launched cultivars with low winter chill requirements onto the market, involving extensive zoning research in recent years. Also, there are species/cultivars with a chilling requirement that can no longer be satisfied in some areas, leading to losses in production/economic inefficiency. Climate change generated significant interest in developing specific tools and models adjusted for each crop. The paper aims to present the available information on apple and pear trees' chilling and heat requirements, focusing on the methods used for their determination. The results reflect comparing the methods used and their efficacy for those species.

Key words*: chilling hours; chilling units; chilling portions; growing degree hours.*

INTRODUCTION

The change in the occurrence of phenophases in fruit trees gave a challenge to climate change. Changes in average temperatures and rainfall and increased short- and long-term extreme events are already affecting crop yields worldwide.

To avoid possible losses, it is necessary to provide helpful information to farmers in real time regarding initiating a particular stage of fruit tree development.

Modern breeding programs have launched cultivars with low winter chill requirements onto the market, involving extensive zoning research in recent years. Also, there are species/cultivars with a chilling requirement that can no longer be satisfied in some areas, leading to losses in production/economic inefficiency.

Climate change generated significant interest in developing specific tools and models adjusted for each crop.

This paper aims to present information on apple and pear trees' chilling and heat requirements, focusing on the methods used for their determination.

RESULTS AND DISCUSSIONS

The apple (*Malus × domestica* Borkh.) and pear (*Pyrus communis* L.) are temperate fruit trees that need a resting period in the winter, correlated with low temperatures.

In this period, fruit trees enter a dormant state when most metabolic processes are temporarily inactivating to avoid chilling injury (Campoy et al., 2011). Dormancy includes endodormancy, when chilling is required, followed by ecodormancy, when budbreak is regulated by environmental factors (Salama et al., 2021; Cornelissen, 2021; Craven, 2022).

Apple and pear trees depend on winter chilling during endodormancy to ensure uniform flowering in spring. In recent years, climate changes led to a decline in winter chill with effects on the fulfillment of the chilling requirements, reducing yield potential and threatening the economic viability of temperate fruit production (Delgado et al., 2021; Craven, 2022; Pertille et al., 2022).

Insufficient winter chilling leads to delayed bud break, decreased flowering and fruit set, and reduced fruit quality.

Apples low chill cultivars have under 800 chilling hours requirement, compared to the 1000-1500 chilling hours (for example, at Golden Delicious), and can be a solution in these regions.
Many breeding programs brought to the market low chill cultivars, for example, apple Anna with less than 300 hours (Hauagge & Cummins, 2000; Cornelissen, 2021), Princesa with less than 450 CU, Baronesa 500-600 CU (Hauagge & Cummins, 2000; Castro et al., 2016).
For pear, low chill cultivars were used, such as Africana, Ayres, Ceres, Flordahome, etc. (Hauagge & Cummins, 2000).
In the tree phenology research history, there were more models for evaluating chilling hours and heat requirements for a specific cultivar from a species. The most used are:

For chilling accumulation

(1) The chilling hours model, which establishes that a cold hour (CH) corresponds to an hour with a temperature value between 0 and 7.2°C (Weinberger, 1950; Richardson et al., 1975; Anderson et al., 1986).
(2) The Utah model is based on quantifying cold units (CU). One cold unit corresponds to one hour for temperatures between 2.5-9.1°C, considered most effective in completing dormancy. Other temperature ranges have 0.5 unity (1.5-2.4°C and 9.2-12.4°C), zero contribution (<1.4°C and 12.5-15.9°C), or negative (>16°C) at rest (Richardson et al., 1974).
There were more extended variants for this model, like the North Carolina model (Shaltout & Unrath, 1983; Anderson & Seeley, 1992), the Positive Utah model (Linsley-Noakes & Allan, 1994), Modified Utah Model (Linvill, 1990), to simulate the local conditions better (Sheard, 2002).
(3) The dynamic model proposes accumulating an intermediate value according to low temperatures that can be reversed by higher temperatures (first stage). Once the value has reached a certain level, cold portions are added permanently, unaffected by higher temperatures (Fishman et al., 1987; Luedeling, 2012; Fadón et al., 2020; Pantelidis & Drogoudi, 2023).
The base variable in all models is the temperature, specifically hourly medium temperature. Special attention is given to local temperatures, which can be registered with data loggers or specific sensors in the meteo stations. More applications have been available for many years for the general public, especially for farmers and all the stakeholders directly affected by local climate conditions.
https://www.ecad.eu/ presents international meteo stations with free daily data for 50-100 years in the past. ECA&D receives data from 85 participants from 65 countries, with 13 elements at 23,335 meteorological stations.

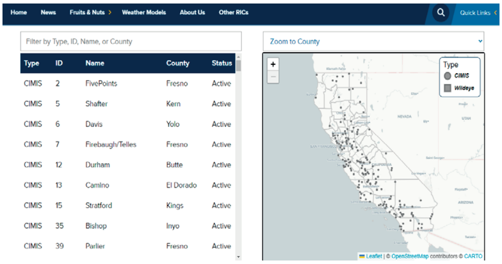

Figure 1. A platform with more meteo stations and applications for stakeholders (source: UCDAVIS)

Other local meteo stations (on site) with free data or not are https://fruitsandnuts.ucdavis.edu/chill-calculator (Figure 1), https://www.meteoblue.com, https://agrometeo.ch/ (Figures 2 and 3), etc.

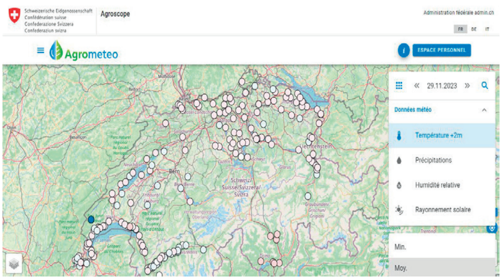

Figure 2. A platform for climatic data with more application for farmers includes (Agroscope.ch)

Temperature can be recorded daily (Tmin, Tmax, Tavg), hourly, every 30 minutes or 15 minutes, etc. In the phenology models, hourly temperature is most used.
The chill units can be determined in a specific region based on the available climatic data and included in historical climatic databases. These are very useful in validating phenology algorithms on a specific species.

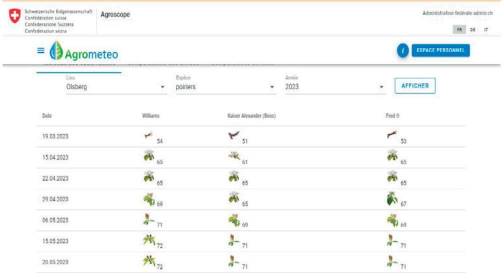

Figure 3. Application for phenology in dynamic (pear) on https://agrometeo.ch/

For heat accumulation

(1) GDD (growing degree days) calculates the days when the average temperature exceeds a certain threshold specific to each species.

GDD = Tavg – Tbase, if Tavg ≥Tbase

GDD = 0 if Tavg < Tbase (McMaster & Wilhelm, 1997).

(2) GDH (growing degree hours) was defined by Richardson et al. (1974) as the amount of energy in the form of accumulated growing degree hours, the number of hours above 4.4°C (40°F). Anderson et al. (1986) defined GDH:

(1) GDH = F*A/2 [1+cos (π + π (TH-TB)/ (TO-TB))], where: TH = hourly temperature, TB = base temperature (4°C for trees), TO = optimal temperature (25°C for trees), TC = critical temperature (36°C in trees), A = TO-TB, F = a stress factor (due to biotic/abiotic factors).

(2) GDH = F*A [1+cos (π/2 + π/2 (TH-TO)/ (TC-TO))]

If TH < TO, equation (1) is used; if TH>=TO, equation (2).

More research was done in the tree phenology modeling, each on a specific area and species, respectively, cultivars.

Luedeling et al. (2021) proposed the PhenoFlex model to predict spring phenology based on the Dynamic Model for chilling accumulation and the Growing-Degree-Hours Model for heat accumulation.

The model was tested on apple (Boskoop grafted on M9 rootstock) and pear (Alexander Lucas grafted on Quince A until 2014, and Quince Adams from 2015) in the Campus Klein-Altendorf, in the experimental orchard of the University of Bonn (6.99°E, 50.63°N).

Miranda et al. (2021) proposed an R package for phenology modeling (fruclimadapt), including functions defined for chill hours(), chill_units(), chill_portions(), GDD_linear(), GDH_linear(), and GDH_asymcur().

A large area of research focused on local cultivars' chilling and heat requirements. Some of the findings are presented below.

Australia (Parkes et al., 2020) - Apple

They used the Chill Hours, Utah, and Dynamic models to assess chilling requirements for apple cultivars. According to their results, they grouped the studied cultivars with low requirements (Cripps Red, Manchurian crab apple), medium (RS103-110, Granny Smith, Cripps Pink, Kalei), and higher chill (Galaxy, Fuji, Hi-Early) classes.

Northwestern Spain (Delgado et al., 2021) - Apple

The local apple cultivars were analyzed using three chill models - the Chilling Hours Model, The Utah Model, and The Dynamic Model. The Growing Degree Hours Model (Anderson et al., 1986) was used as a heat accumulation model. Clara, Coloradona, Perezosa, Verdialona, Blanquina, De la Riega, Teorica, Xuanina, and Collaos were under 1300 CU, while Perico and Raxao were 1495 CU.

Winchester, VA, USA (Sapkota et al., 2021) - Apple

The endodormancy release for the two cultivars was achieved after accumulating 1000 CH. Ecodormancy release of Cripps Pink occurred at 3000 GDH and 4000 GDH at Honeycrisp cultivar.

Brazil (Pertille et al., 2022) - Apple

Gala and Fuji were analyzed in three regions with three chilling hours models and GDH for heat accumulation. The chill and heat requirements were different according to region and cultivar.

Fluvià river lower course subbasin, NE Spain (Funes et al., 2016) - Apple

Chilling and heat requirements for Brookfield Gala, Granny Smith, Fuji Chofu, Golden Smoothee, Early Read One, Red Chief, Aporo, and Golden Reinders ranged between 62.5-68.4 CR and 9229.7-10272.5 HR.

USA, the Pacific Northwest region (Noorazar et al., 2020) - Apple

Golden Delicious (50 CP) and Gala (50-55 CP) were at risk for insufficient chill accumulation.

North-eastern Belgium (Drepper et al., 2020) - Apple and pear

Four pear cultivars (Conference, Durondeau, Doyenné, and Triomphe) and four apple cultivars (Jonagold, Golden Delicious, Boskoop, and Cox Orange) were analyzed. Two models were used, including the Dynamic + GDH Model.

Australia (Parkes, 2017) - Apple and pear

They included climate change scenarios for pome fruit in 2030 and 2050, including the likely impact of climate change on winter chill and extreme heat.

The outcomes included historical trends in average temperatures, winter chill and heat days, climate projections for average winter chill and heat days, relationships between temperature and flowering (Dynamic, Utah, and Chill Hours were used), potential impacts of warming temperatures on flowering, options for adaptation: management of flowering under future climates, relationships between summer temperatures and the incidence of sunburn under net and no net.

Ethiopia (Melke & Fetene, 2014) - Apple

Description of the apple production and characteristics. Challenges identified included a lack of adequate chilling temperature.

KwaZulu-Natal, South Africa (Sheard, 2002) - Apple and pear

Utah chill unit model, Daily Positive Utah Chill unit model (DPCU), and Dynamic model were used. For apples, DPCU was determined for Royal Gala (800-1000+), Golden Delicious (800-1000+), Granny Smith (600), Braeburn (800), and Fuji (800-1000). For pear Bon Chretien and Golden Russet Bosc had 800-1000+ DPCU, Forelle 600-700, Rosemarie 700-800, Flamingo 700-900 DPCU.

Brazil (Carvalho et al., 2014) - Apple and pear

The research evaluated the dormancy dynamic of Imperial Gala apple tree buds and Hosui pear tree buds in a region of low chill occurrence.

Belgium (Drepper et al., 2022) - Pear

The study focused on identifying the spatiotemporal trends of flowering, spring frost, and their co-occurrence in Belgium at the pear and comparing the effectiveness of a set of recursive bias correction methods.

Subtropical climate (Verma et al., 2010) - Apple and pear

A collection of data for chilling accumulation and cultivars needs for different subtropical regions.

Romania (Chițu & Păltineanu, 2020) - Apple and pear

The research evaluated the effects of climate change in apples and pears on four phenological stages: bud swelling (BBCH 51), budburst (BBCH 53), beginning of flowering (BBCH 61), and end of flowering (BBCH 69).

Pacific Northwest (Houston et al., 2020) - Apple and pear

Identifying the chilling hour requirements and approximate hardiness zones for several apple and pear cultivars.

CONCLUSIONS

Apple and pear are well-studied fruit species, including the correlation between phenology and climate change. Besides diversified models applied to quantify the chilling and heat requirements, more cultivars are well known for their chilling and heat needs to complete a phenological stage.

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EVALUATION OF CHILLING AND HEAT REQUIREMENTS OF PAW-PAW (*ASIMINA TRILOBA* L. DUNAL) AND JUJUBE (*ZIZIPHUS JUJUBA* MILL.) (REVIEW)

Ana Cornelia BUTCARU, Andrei Florin TABACU, Rareş Cosmin PÂNDARU, Cosmin Alexandru MIHAI, Adrian ASĂNICĂ, Florin STĂNICĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mcosminalexandru@yahoo.com

Abstract

Climate change is a reality of the difficult period we are going through. Plant evolution is generally strongly correlated with variations in temperatures, precipitation, and solar radiation being affected by extreme events. Late frosts influence fruit production, the number of cold hours (perhaps insufficient in recent years), and winter windows in which very high temperatures occur and negatively influence the dormancy of some fruit-bearing species. Paw-paw (Asimina triloba L. Dunal) and jujube (Ziziphus jujuba Mill.) are new fruit species for Romanian areas more resilient to climate change. This paper aims to present the available information on this new fruit species' chilling and heat requirements, focusing on the methods used for their determination. The results reflected the scarcity of research on these fruit species and the necessity to have a methodology with the possibility to extend to new cultivars in different areas.

Key words*: chilling hours; chilling units; chilling portions; growing degree hours.*

INTRODUCTION

Climate change is a reality of the difficult period we are going through. Plant evolution is generally strongly correlated with variations in temperatures, precipitation, and solar radiation being affected by extreme events. Late frosts influence fruit production, the number of cold hours (perhaps insufficient in recent years), and winter windows in which very high temperatures occur and negatively influence the dormancy of some fruit-bearing species. Paw-paw (*Asimina triloba* L. Dunal) and jujube (*Ziziphus jujuba* Mill.) are new fruit species for Romania areas more resilient to climate change. This paper aims to present the available information on this new fruit species' chilling- and heat requirements, focusing on the methods used for their determination.

The timing of life-history events, or phenology, can reveal information about species' types and levels of interactions with their surroundings. For instance, a plant's ability to compete for light and its susceptibility to disease or cold are influenced by phenology, which is the time of leaf emergence, expansion, and abscission (Lechowicz, 1984; Sun et al., 2006).

According to Rosenzweig et al. (2008), long-term phenological investigations conducted at the species level and particular places can offer concrete proof of the effects of climate fluctuation and change. Global reports have shown that plant phenology has apparent responses to climate change. Despite these crops' significant economic and agricultural values, relatively little research has been done on how fruit trees respond to climate change, with most published studies concentrating on phenological changes in natural vegetation (Chmielewski et al., 2004).

According to Legave et al. (2008), fruit trees' flowering phenology significantly affects fruit set, pollination, and yield. Interest in how climate change affects fruit blossoming has grown globally. According to studies conducted by Chmielewski et al. (2004), Grab & Craparo (2011), Guédon & Legave (2008), Legave & Clauzel (2005), and Wolfe et al. (2005), global warming has caused various fruit trees to flower earlier in numerous regions. Nonetheless, delayed blossoming has been observed in certain fruit trees planted in regions significantly warmer than their natural habitat (Elloumi et al., 2013; Legave et al., 2013). The effect of climate change on plant dormancy is most likely the cause of flowering's timing.

Asimina triloba (L.) Dunal, commonly known as paw-paw, is the only member of the Annonaceae family growing in temperate zones and, in particular, grows wild in the eastern United States, ranging from northern Florida to southern Ontario (Canada) and as far west as eastern Nebraska. Today, it is found in temperate climate countries (i.e., Italy, China, Japan, Israel, Belgium, Portugal, and Romania). There are over 60 varieties cultivated in the world, which differ in trunk diameter tree, fruit size, skin and pulp color, fruit flavors, and ripening periods (Pomper & Layner, 2005; Brannan & Coyle, 2021).

Paw-paw has few disease problems thanks to the high content of acetogenins in roots, twigs, bark, immature fruits, and seeds (Ratnayake et al., 1992; McLaughlin, 1997).

Research suggests that paw-paw fruit pulp has the potential to be added to various consumer goods to add increased nutritional benefits or flavor enhancement (Brannan et al., 2012) (Lolletti et al., 2021)

Figure 1. *Asimina triloba* flowers

Ziziphus jujuba Mill. belongs to the family Rhamnaceae, and more than 170 species are in the *Ziziphus* genus.

Figure 2. *Zizizphus jujuba* flowers

The jujube tree is mainly distributed in the subtropical and tropical regions of Asia, Russia, northern Africa, southern Europe, the Middle East, and the southwestern USA. (Hernández et al., 2014). Furthermore, it is well-known for its highly nutritive fruits.

RESULTS AND DISCUSSIONS

Asimina (*Asimina triloba* L. Dunal)

Asimina is well adapted to different climatic zones and requires a minimum of 400 annual chill units, 160 frost-free days, and 80 cm of annual precipitation, with most falling during spring and summer (Peterson, 1991).

Abu-Asab et al. (2001) integrated the observation from more than 125 persons, who contributed with records for first-flowering dates through the years for 100 species, representing 44 families of angiosperms, for 29 years of the 30 years 1970-1999 (1984 not recorded) in the Washington, DC, area. *Asimina triloba* is included in the list.

Crabtree (2004) evaluated GDDs required for fruit ripening ranging from 2200 to 3200. Between the studied populations, the growing degree of days required for fruit ripening was not found to be significantly different. However, GDDs varied significantly between accessions, with trees from New York requiring the fewest GDDs to ripen, 2262, and the other five populations requiring a similar number of GDDs (2400-2500) for ripening. Growing degree days (GDDs) were calculated using a base temperature of 10°C, and the beginning degree day accumulation was on May 15, which was approximately the end of flowering (http://wwwagwx.ca.uky.edu/calculators.html).

Pomper et al. (2008) studied the flowering and fruiting characteristics of Middletown, Overleese, PA-Golden, Sunflower, Wells, Wilson, and NC-1 cultivars. The following results were obtained: Flowering peak and duration were not correlated with the growing degree days; Growing Degree Days from the first flower to the peak flower were similar in 2004 and 2006 and were significantly lower in 2005. The GDD from flower peak to harvest peak were fewer for 2004 than for 2005 and 2006, indicating that these later years had warmer temperatures than 2004. The number of days was similar for the tested cultivars.

Growing degree days (GDDs) were calculated using a base temperature of 10°C (http://wwwagwx.ca.uky.edu/calculators.html). Szilagyi et al. (2016) studied the flowering of Asimina in the northern part of Romania. It was noted that at the beginning, the button phase lasted three days, and the ornamental potential of the flowers, given by floral decoration that displays itself until the fall of the corolla, lasted 24 days (in 2016). The end of the floral decoration, marked by the fall of the corolla, lasted three days (April-May).

Bivariate model partial dependence graphs indicate that pawpaw is compatible with the warmest circumstances in the study region, with mean annual temperatures >9.0°C showing a significant increase in compatibility. It should be noted that the study area has a strong link between temperature and precipitation variables. This means warmer regions receive less precipitation; for instance, an average annual temperature of 9.0°C typically translates to ~44 cm of growing-season precipitation (May-September) (Tulowiecki, 2020).

Figure 3. *Asimina triloba* fruits

Jujube (*Ziziphus jujuba* Mill.)

Mishra & Krška (2008) studied to find a suitable base temperature for different phenological stages in *Ziziphus jujuba* Mill. They use temperatures above 7, 9, and 11 °C as threshold values for the phenology of jujube. 11°C was found to be the most suitable base temperature for jujube.

Du (2009) studied the chilling requirements of different jujube cultivars correlated with the changes in carbohydrates during dormancy. The cultivars were clustered according to their chilling needs, from 399 C.U. to 580 C.U. It also presented a method of identifying dormancy by measuring the changes in total soluble sugar and starch contents.

Guo et al. (2014) studied PLS regression between phenological dates, daily chilling, and heat accumulation, which can be used efficiently to identify chilling and forcing periods and to estimate the chilling and heat requirements of temperate trees provided long-term temperature and phenology data available. The forcing periods for jujube began after half of the chilling requirements were met. During the times shown to be significant for heat accumulation and cooling, rates of both were impacted by climate change. The heat buildup of jujube has increased by 92.3 GDH annually during the last 50 years. Although there was a tendency for winter cold to rise, this trend was not significant enough to rule out the null hypothesis that there was no change over time.

Jujube flowering times were dictated by heat accumulation, with a minor influence from cold buildup on bloom timing.

Zou et al. (2017) presented the time development for the jujube bud flower of Jinsi No. 4 in Hunan between 2010 and 2011. Pre-differentiation started on April 9th for 14 days, continuing with initial differentiation (3 days), sepal differentiation (3 days), petal differentiation (2 days), stamen differentiation (2 days), pistil differentiation (2 days), alabastrum (20 days), alabastrum break (2 days), sepal flattening (2 days), petal flattening (2 days), stamen flattening (2 days). Filament withering (2 days), ovule swelling (3 days), early flowering period (11 days), full blossom period (15 days), and the end of flowering period (16 days).

In a study by Krishna et al. (2018), Growing degree days were calculated following Mendes et al. (2017) for a base temperature of 4°C. They analyzed the phenological growth stages of Indian jujube (*Ziziphus mauritiana* Lamk.) according to the BBCH scale (Table 1).

Table 1. Phenological growth stages of Indian jujube and GDDs (source Krishna et al., 2018)

BBCH	Substage	Duration	Degree days
0	00	35	1,143.70
	01	6	166.2
	03	5	143.4
	07	4	113.9
	09	3	85.3

BBCH	Substage	Duration	Degree days
1	10	2	56.7
	11	1	30.8
	12	1	31.2
	13	1	30.4
	14	1	29.9
	15	1	32.7
	16	1	29.6
	19	2	59.5
3	31	8	228.6
	32	7	198.3
	33	6	168.5
	34	6	198.9
	35	5	125.7
	36	4	101.1
	37	5	129.1
	38	3	83.5
	39	4	110.2
5	51	16	430.1
	54	5	135.6
	56	5	132.6
	59	4	109.5
6	60	3	83.1
	61	2	52.9
	62	3	75.6
	63	3	75.6
	64	2	50.1
	65	2	52.4
	67	4	101.2
	69	3	76.7
7	71	9	224.7
	72	13	287.4
	73	8	166.9
	74	9	152.8
	75	14	155.5
	76	15	218.9
	77	17	166.4
	78	16	195.4
	79	8	83.8
8	80	4	36.3
	81	3	27.6
	85	3	26.3
	88	4	45.9
	89	15	189.9
9	91	35	640.1
	93	10	229.8
	95	10	256.3
	97	15	406.8
Total accumulated degree			8,183.40

The chilling requirement of the Zhanshanmizao cultivar was studied and presented by Deng et al. (2018). The preliminary calculation showed that the chilling requirement was 494 h, according to the 0-7.2°C model.

Bai et al. (2019) proposed a different model to assess the correlation between phenology stages and temperatures in their study regarding the WOFOST model in simulating jujube fruit tree growth under different irrigation regimes.

According to the study by Gao et al. (2021), the diploid Dongzao cultivar had higher levels of cold tolerance than autotetraploid due to its morphological and physiological analysis.

Figure 4. *Ziziphus jujuba* riped fruits

Chițu et al. (2022) presented a model-based assessment of Romania's climatic suitability for extending new fruit species, including jujube crops, considering the 100-180 growing days.

It was found that, from the point of view of climatic suitability, jujube trees present reduced restrictions in most areas of the country. In Romania, according to their study, *Ziziphus jujuba* Mill. Species are restricted only in areas where the temperature drops below -23°C.

CONCLUSIONS

The results reflected the scarcity of research on the phenology of these fruit species correlated to chilling and heat requirements and the necessity for a methodology that could extend to new cultivars in different areas.

ACKNOWLEDGEMENTS

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A DIGITAL SYSTEM TO MONITOR THE CANOPY IN SOME APPLE AND QUINCE CULTIVARS

Ana Cornelia BUTCARU, Doru Ionel DUMITRAȘCU, Cosmin Alexandru MIHAI, Florin STĂNICĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mcosminalexandru@yahoo.com

Abstract

Nowadays, we still need to know every link in the production chain to optimize it by reducing costs and allocating resources to develop essential segments. WinSCANOPY is a system that analyses tree canopy through image analysis. This work aims to present the influence of the planting system and the fruit tree species on specific canopy parameters (foliar index, direct, diffuse, and total radiation) by monitoring the canopy projection in an apple and quince orchard. A secondary objective consisted of studying the canopy developing dynamic during a growing season in the analyzed species. The results compare the parameters monitored on the four sides of the trees (North, East, South, and West) in three moments of the day and five series in the vegetable season.

***Key words**: non-destructive equipment, foliar index, direct, diffuse, and total radiation*

INTRODUCTION

Modern analysis methods offer new possibilities to understand the interaction of the fruit tree with the environment and its response to orchard management.

The paper aims to present a method to analyze the influence of the planting system on some apple and quince cultivars using several canopy parameters. Foliar index, direct, diffuse, and total radiation were measured. At the same time, the canopy dynamic during a growing season in the analyzed species was monitored.

In modern horticulture, selection, selective crosses, and system planting designed for super-intensive orchards led to yields of more than 80 tonnes per hectare in the apple crop, aided by modern cultivation technologies: controlled fertilization (fertigation), foliar fertilization, phytosanitary protection, and the use of different canopy shapes that promote the obtaining of fruits and the most judicious use of the less and less available labor force for carrying out maintenance and harvesting works (Ghena et al., 2004; Stănică & Peticilă, 2011).

In the last decades, we have gone from natural selection and trees with natural and massive crowns, challenging to harvest, which are still found in nature today, to controlled selection and breeding, with clear objectives and the creation of shape canopies adapted to the demands of society of today, especially maximum production with minimum labor consumption.

From extensive orchards, we have moved to a plantation with more than 3,300 plants per ha, and the first step was the pyramid canopy. It was still a canopy for the vigorous plants. Still, it allowed better harvest management due to the architecture, the skeleton being well defined, strong branches, and the majority of the fruits towards the outside due to the shading effect of the axis. The pyramidal canopy shape was a canopy that delayed fruiting, requiring a long time to achieve it at planting densities (5/4 or 6/5 m). It was a canopy intended for extensive orchards with vigorous cultivars and rootstocks.

The pyramid canopy shape was used and promoted until around the 1950s when the Vase canopy was discovered, which resembled the pyramid's first floor. This was formed by removing the spindle after the branches had consolidated their position. The advantages of the Vase system planting consisted of redistributing the vigor of the pyramid's vertical axis on the three or four arms of the Vase canopy. This fact allowed the fruiting branches to be closer to the ground, simplifying pruning and harvesting and much better penetration of light inside the crown, reducing the pressure of fungal diseases that develop in an environment with increased humidity and light radiation.

From this point, a step forward was the flattened crown forms: Late Flattened Vase, Simple Palmette, Tiered Palmette, Non-tiered Palmette, Free Palmette, etc., which increased the number of trees per hectare and brought the fruit closer to the soil, implicitly facilitating harvesting and maintenance work (pruning, harvesting, phytosanitary protection).

The next step towards orchards with high-yielding planting systems was the discovery, creation, and use of low-vigor rootstocks, which further increased the number of trees per hectare and led to a change in tree crown architecture to simpler, more productive, and more accessible and faster to create.

Obtaining the Thin Spindle required removing only a few pyramid levels and replacing them with semi-skeletal, fruit-bearing branches. A simple crown, in which only a few elements represent the skeleton, was the zig-zag axis, formed by replacing the arrow extension with the competitor or the next branch, provided that a relatively straight and vertical line of growth was kept.

From an evolutionary point of view, the following canopy shapes further simplified the formation and creation of fruit system planting, to be created by a workforce with as little experience and training as possible. Simplicity increased workforce efficiency.

The next canopy shape was the Vertical Axis canopy shape. This was the simpler version of the Slender spindle canopy. The skeleton (the elements that remain in the architecture of the tree throughout its lifetime) was represented only by the trunk and its extension (axis), plus the skeletal branches that were periodically replaced (the fruiting branches) by minimal cuts. This canopy shape speeded up fruiting. For planting trees with early shoots (at least in the case of apples), the first fruits were obtained from the very first year after planting. An economical production could be obtained from the second or third year.

The canopy, which, instead of one axis, had two parallel axes of identical force at the same distance from the trunk, with the same branching angle between them, was called Bi-Baum® (in German, "two trees"). This canopy type could theoretically reduce the cost of planting material by 50%. Practically, these expenses were increased by the longer formation time of this canopy in the nursery or the newly established plantation.

In addition to the disadvantage of a more extended formation and fruiting time, the Bi-Baum® crown had the advantage of a longer lifespan compared to the Vertical Axis and the minor need for trees to establish a plantation.

The Trident canopy was formed similarly to the Bi-Baum®, with the difference that it had three instead of two central arms. So, this planting system had the advantage of reducing the need for planting material to a third of the Vertical Axis. (Hoza, 2000; Grădinariu, 2002; Hoza, 2003; Ghena & Braniște, 2003; Iordănescu, 2008; Stănică & Braniște, 2011; Asănică & Hoza, 2013; Sumedrea et al., 2014; Cimpoieș, 2018).

At the farm level, there is a need to thoroughly know every link in the production chain to optimize it by reducing costs and allocating resources to develop essential segments. Growing fruit trees, although a storied activity, still has aspects we do not fully understand or have yet to measure.

WinSCANOPY system analyses tree canopies through indirect methods and image analysis.

Modern analysis methods offer new possibilities to understand the interaction of the tree's canopy with the environment and their response to the applied culture technology. These observations lead to the efficiency of culture technology and the creation of more efficient canopy shapes that require as little input as possible for formation and maintenance and give a qualitative and quantitatively efficient production with a reduced labor requirement.

The main objective of the research was to determine the influence of the crown shape and the tree species on specific parameters (foliar index, direct, diffuse, and total radiation, etc.) by using the WinSCANOPY system for monitoring the canopy projection in some tree species.

A secondary objective was monitoring how the analyzed species' canopy shapes developed during a growing season.

MATERIALS AND METHODS

The experiment was conducted in the Didactic Experimental Field of the Faculty of Horticulture, located north of the city, in the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The representative profile has the geographical coordinates 44°28'10" N and 26°04'00" N.

Four canopy shapes were analyzed for the biological material: Vertical Axis, Bi-Baum®, Trident at apple, and Vase at quince.

WinSCANOPY system for monitoring the crown projection was used (WinSCANOPY, 2014). The system includes image acquisition hardware, a fisheye lens camera, and computer programs for hemispheric and covers image analysis and data visualization.

During these analyses, it was possible to observe:

• Offset fractions per sky region, altitude ring, and direction. Opening on the lifting ring.

• The level of direct and indirect (diffuse) solar radiation above and below the crown and total radiation throughout the day.

• The angle of the leaves in the crown.

• Distribution and duration of sunny areas at crown level.

• Crowding index according to zenith angle and sky region.

• The leaf projection coefficient depends on the zenith angle.

• Distribution of measured and theoretical shaded areas at crown level.

The method consisted of taking several photographs and then analyzing them with the WinSCANOPY software. The pictures were taken in the phenophase of intensive shoot growth in 5 series with repetition at ten-day intervals. The device has a series of rings that allow it to balance perfectly horizontally, with the objective towards the zenith, or perfectly downwards if a series of weights are placed on it with which it is provided.

During the same day, three series of photos were taken in the morning, around 9 o'clock, at noon, when the sun was at its zenith, around 2 o'clock, and in the evening at 6 o'clock. The trees were also photographed (from bottom to top) from each cardinal point, resulting in a total of 60 pictures for each tree (4 cardinal points x 3 moments of the day x 5 series) (Figures 1 and 2).

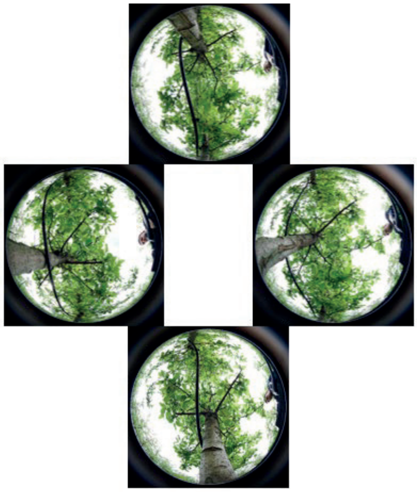

Figure 1. Apple - Vertical Axis canopy shape, series 1, morning (source: own data)

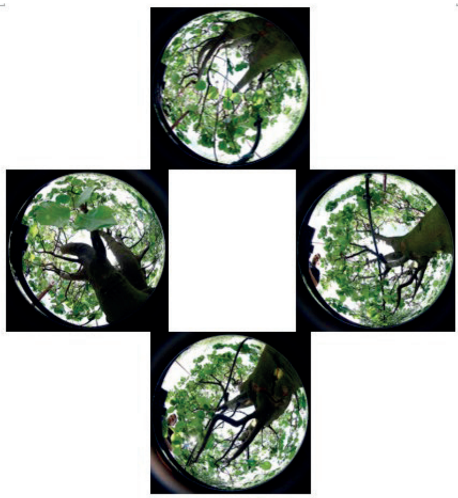

Figure 2. Quince - Vase canopy shape, series 1, morning (source: own data)

For the descriptive statistics of the data, XLScanopy software, Microsoft Excel 2016, and IBM SPSS v. 28.0.1.1 were used for a significance level of $p = 0.05$.

RESULTS AND DISCUSSIONS

The results quantified the canopy projection on the ground for all experimental variants.

Influence of species and planting system on crown projection

Gap fractions (% Cer_1) and crown openness (% Cer_2) were rendered dynamically for the two species and canopy shapes analyzed (Figures 3-6).

In the quince, in the Vase planting system (Figure 3), the East, North, and South showed similar values, decreasing as the crown developed, varying between 27.09% - 13.46%.

The analyzed data showed how the western part of the canopy was more developed, the parameters varying between 15.79%-6.45% in the morning, 19.3%-8.29% at noon, and 10.99-6.82% in the evening.

The values determined in the measurements taken at noon were the highest, followed by those in the morning and evening.

In the apple species, in the Vertical Axis system planting, the values were between (1) 28.72-12.74% (East), 32.62-14.28% (North), 33.68-15.43 % (South), and 37.36-15.33% (West) as well as (2) 38.75-13.03% (East), 39.02-15.15% (North), 38.98-14.43% (South), and 43.43-16.02% (West) (Figure 4).

The Bi-Baum® system resulted in the following values: 36.29%-15.48% (East), 36.76%-14.44% (North), 41.99-15.6% (South), and 35.66-13.55% (West) (Figure 5).

In the Trident canopy shape, the values were between 30.16-9.83% (East), 22.28-8.02% (North), 32.26-9.91% (South), and 29.08-9.69% (West) (Figure 6).

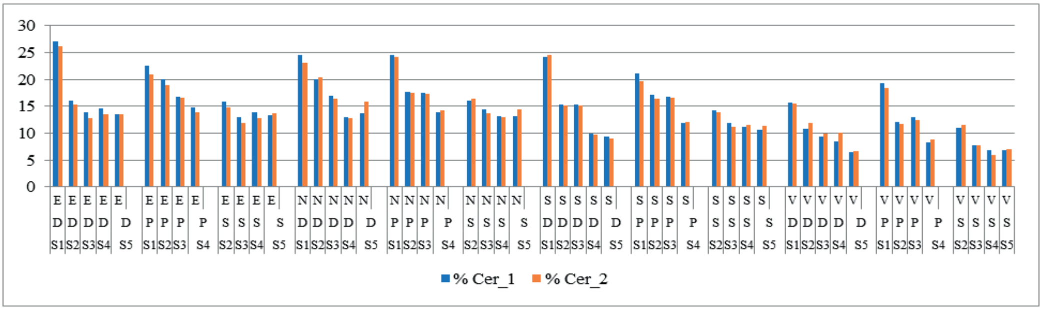

Figure 3. Vase Canopy projection to quince (%) (source: own data)

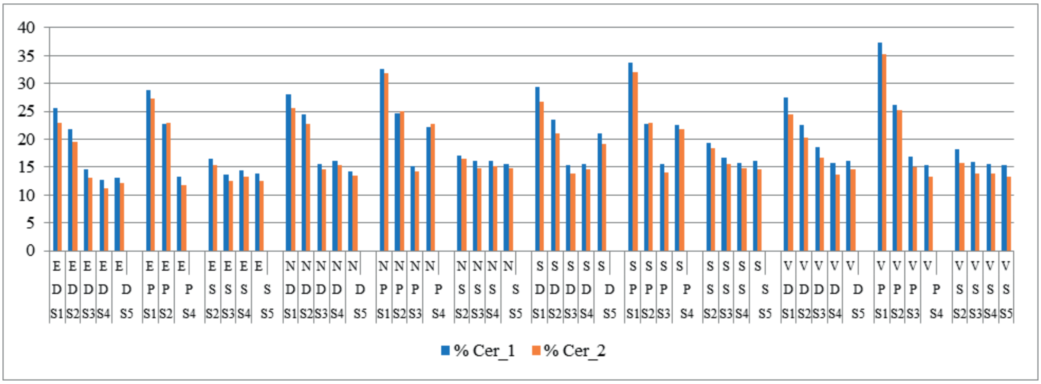

Figure 4. Canopy projection Vertical Axis (1) to apple (%) (source: own data)

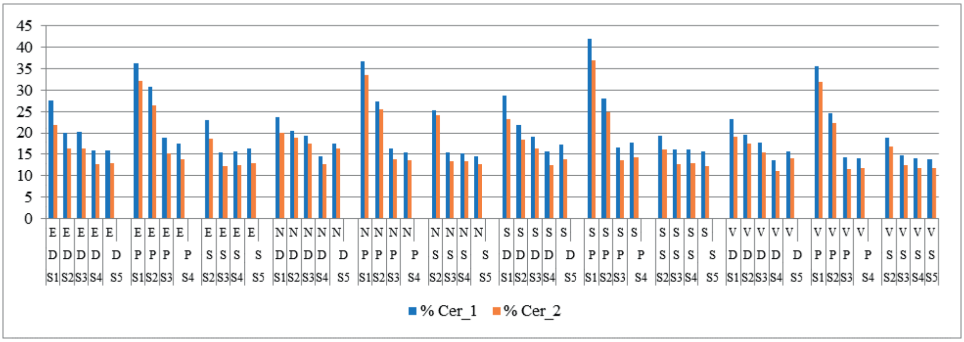

Figure 5. Projection of Bi-Baum® canopy shape at apple (%) (source: own data)

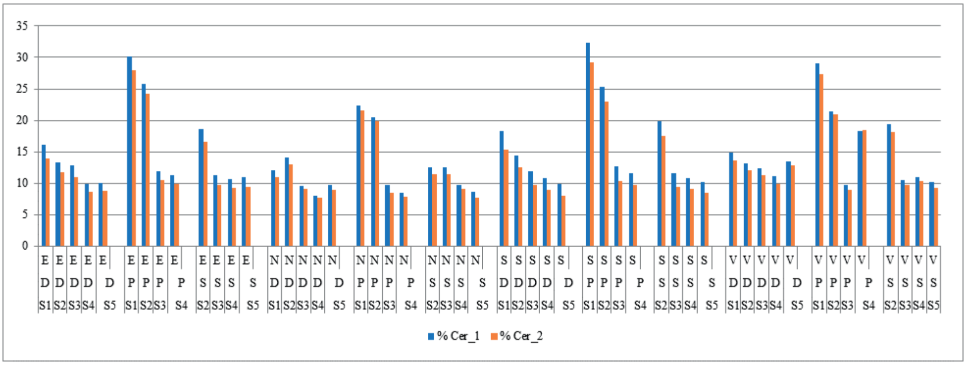

Figure 6. Projection of Trident canopy shape at apple (%) (source: own data)

The influence of species and canopy shape on the evolution of the Foliar Index

The foliar index varied depending on the cultivar and planting system/canopy. From Figure 7, for quinces, Vase canopy shape, significant differences in the index could be observed between the four cardinal points. The growth tendency was present, natural to crown development. Values ranged from 1.34-2.11 (East), 1.17-1.91 (North), 1.08-2.53 (South), and 1.78-3.69 (West).

In apple, at the Vertical Axis canopy shape the following values for the Foliar index (1) 1.09-2.03 (East), 0.76-1.53 (North), 0.78-1.5 (South), and 0.76-1.76 (West), respectively (2) 0.64-1.74 (East), 0.74-1.72 (North), 0.59-1.45 (South), and 0.67-1.73 (West) were presented (Figure 8).

In the Bi-Baum® canopy shape, the Foliar Index showed values between 0.94-1.83 (East), 0.63-1.6 (North), 0.9-2.56 (South), and 0.94-1.98 (West) (Figure 9).

The Foliar index in the Trident system planting had values between 1.31-2.74 (East), 1.03-2.14 (North), 1-2.87 (South), and 0.93-2.04 (west) (Figure 10).

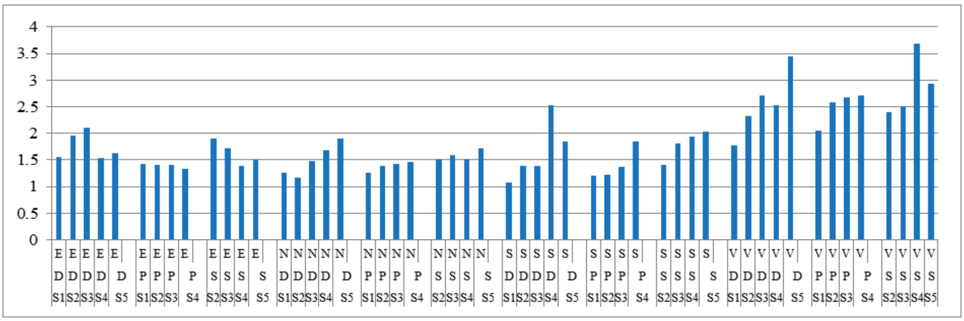

Figure 7. The evolution of the foliar index in quince to the shape of the Vase canopy shape (source: own data)

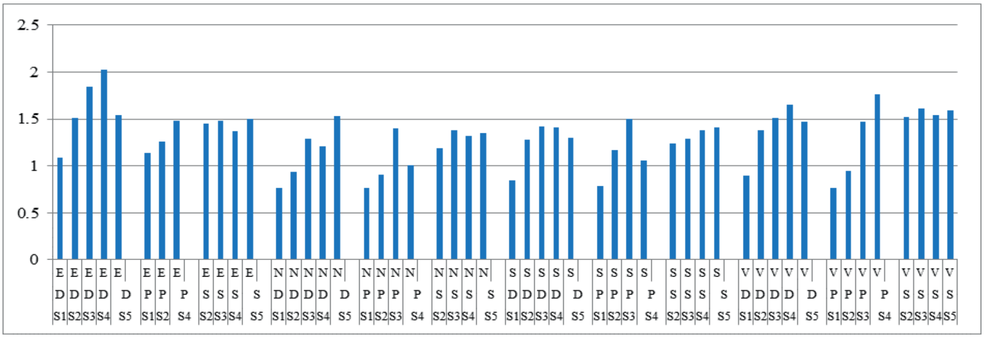

Figure 8. The evolution of the leaf index in apple, Vertical Axis 1 (source: own data)

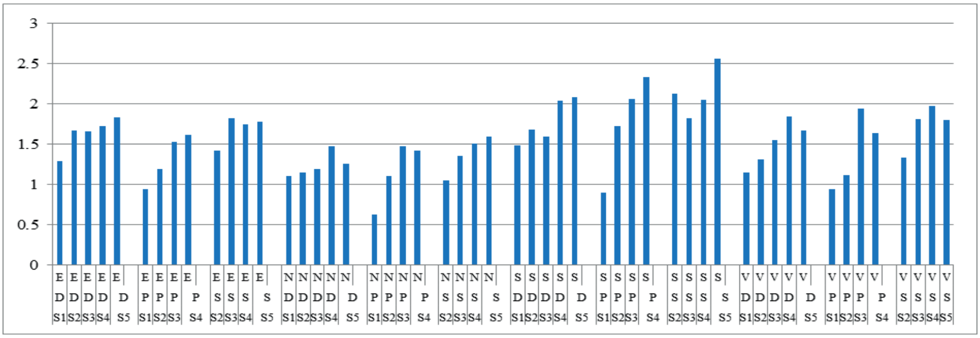

Figure 9. The evolution of the leaf index in apple, Bi-Baum (source: own data)

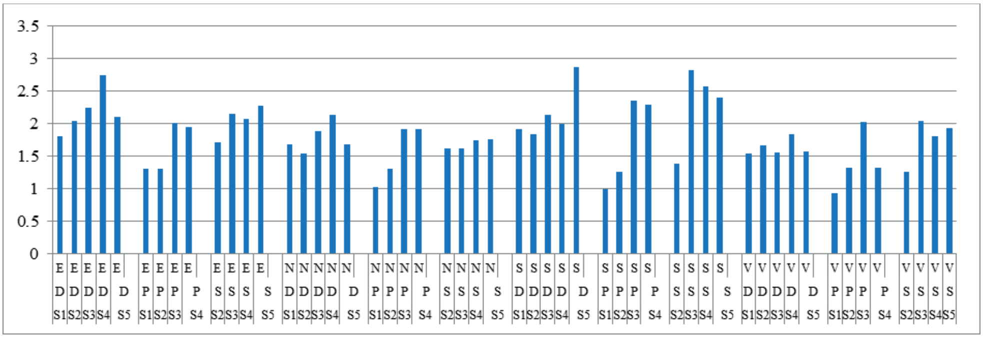

Figure 10. The evolution of the leaf index in apple, Trident (source: own data)

The influence of species and canopy shape on the level of direct and diffuse solar radiation at the tree level

Depending on the tree's growing stage, a decrease in direct and diffuse radiation values was observed under the tree's canopy, a direct cause being the increase in vegetative mass (Figure 11). Species and canopy shape had essential influences on the variation in these factors, briefly outlined below.

In quince, Vase canopy shape, total radiation decreased between the initial and final time of the experiment as follows: (East) 10.66-3.14 MjorMol/m^2/day with an average of 81% direct radiation and 19% diffuse radiation (North) 12.43-1.63 MjorMol/m^2/day with an average of 76% direct radiation and 24% diffuse radiation, (South) 9.72-2.46 MjorMol/m^2/day with an average of 82% direct radiation and 18 % diffuse radiation, (West) 8.25-0.64 MjorMol/m^2/day with average of 71% direct radiation and 29% diffuse radiation (Figure 11).

The apple, on the canopy shapes analyzed, presented the values summarized in Table 1.

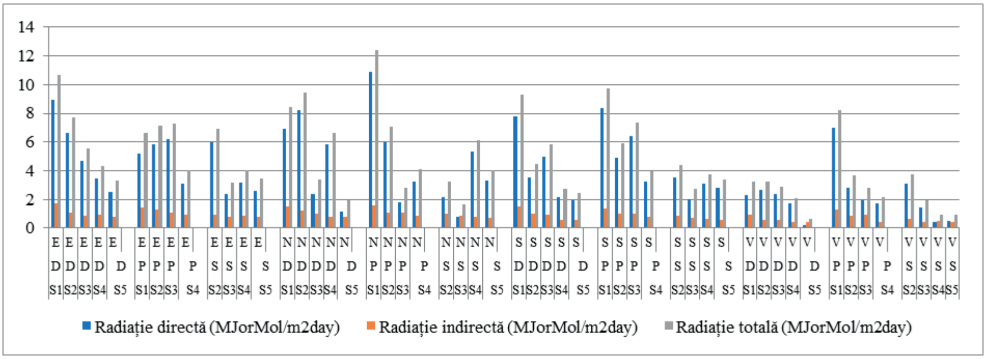

Figure 11. The level of solar radiation under the Vase canopy at quince (source: own data)

Table 1. The influence of the canopy shape and the orientation to the cardinal points on the solar radiation in apple

Cardinal point	Vertical axis 1 Total radiation (MjorMol/m^{2}day)		Vertical axis 2 Total radiation (MjorMol/m^{2}day)		Bi-Baum Total radiation (MjorMol/m^{2}day)		Trident Total radiation (MjorMol/m^{2}day)	
	max direct%	min diffuse%	max direct%	min diffuse%	max direct%	min diffuse%	max direct%	min diffuse%
	14.81	4.31	17.45	4.48	16.82	7.58	13.44	4.2
East	85	15	84	16	87	13	86	14
	16.47	7.03	23.58	6.32	18.63	6.54	10.23	3.74
North	89	11	87	13	88	12	87	13
	20.14	7.82	18.46	6.5	19.3	6.77	15.65	2.13
South	90	10	87	13	87	13	81	19
	15.84	6.84	18.03	5.03	19.75	4.19	19.37	1.96
West	87	13	85	15	83	17	76	24

CONCLUSIONS

Following the research, we recommend the WinSCANOPY system as an extremely useful tool for monitoring crown parameters in fruit growing.
The method can be applied and extended to more species. The results were significant for optimizing technical decisions regarding pruning and other tree management actions and for deeper analysis of the tree physiology.

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SWEET CHERRY FRUIT CRACKING - A CHRONIC PROBLEM IN THE ERA OF CLIMATE CHANGE

Mihai CHIVU[1, 2], Mădălina BUTAC [1], Adrian ASĂNICĂ[2]

[1]Research Institute for Fruit Growing Pitesti-Mărăcineni, 402 Mărului, Mărăcineni, 117450, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihai_chivu7915@yahoo.com

Abstract.

Scientific and practical interest in sweet cherry fruit cracking has steadily increased in the last decades, due to the higher incidence caused by climate changes. The problem is still less understood and the management strategies to prevent the incidence of the disorder in susceptible cultivars as well. Hailstorms and heavy rainfall after a long period of drought which are associated with new manifestations of climate changes might increase fruit cracking phenomenon. Other factors related to fruit characteristics or some cultural practices are also influencing fruit cracking. The responses of the trees to the application of some compounds (minerals, anti-transpirants and growth regulators) just before harvesting, vary according to the cultivar, application time, concentration and their type, which makes it difficult to generalize their effects. Moreover, their effectiveness it is not high and sometimes even counterproductive. Protecting crops with macrotunnels or covering orchards with polyethylene films, the use of seaweed-based biostimulants or the mechanical removal of rainwater have proven to be quite effective in many situations, but the need to develop new strategies to mitigate fruit cracking requires extra information on the mechanisms leading to skin cracking. Many studies have shown that some varieties manifest a high resistance to cracking, and phenotyping for selection the most resistant genotypes can be correlated with new molecular research and findings of molecular markers associated with fruit cracking, and also of the genes involved in the formation of the cuticle and cell wall. Current review explores the factors which are contributing to fruit cracking in sweet cherry, report advances recommended measures to reduce this disorder, and indicate directions for future research.

***Key words**: climate changes, fruit cracking, rainfall, sweet cherry.*

INTRODUCTION

Sweet cherries are extremely sensitive to cracking, most of the time with severe effects on commercial production. Chrinstensen (1996) showed that if only 25% of the fruits are affected, the harvest becomes uneconomical, moreover even in a smaller percentage the fruits are quickly attacked by microbial agents, which makes them undesirable for marketing, becoming at the same time a source of infection in the orchard.

Considering that the phenomenon occurs before or after rain shortly before harvest, it is considered that fruit cracking occurs due to the water uptake (either osmotic, through the epidermis, or vascular, through the pedicel), which results in increase fruit turgor, volume and area surface, up to a critical value that causes the fruit to crack - "*critical turgor pressure concept*" (Considine and Kriedemann, 1972; Measham et al., 2009; Sekse, 1995; Sekse et al., 2005).

The difference in the osmotic potential of the water inside the parenchyma cells of the mesocarp and the water on the surface of the fruit's skin (usually close to zero) sets in motion the necessary force for absorption by osmosis of water from the outside into the inside of the fruit (Grimm et al., 2020).

In short, the theoretical model for sweet cherry fruit cracking agreed most of the time implies the acceptance of several aspects:

- plasmolysis of the epidermal cells exposed to mesocarp juice due to the potential difference between them (Grimm et al., 2015);
- in phase of rapid growth, most of the water and nutrients reach through the phloem in the vascular system of the fruit (Brüggenwirth et al., 2016) oriented primarily to the mesocarp and the ovules and less to the epidermis (Grimm et al., 2017).

Microcracks, usually detectable under a light microscope, affect the cuticle and usually do not assume damage to the mechanical properties of the fruit skin (Brüggenwirth et al., 2014), while **macrocracks** can reach the epidermal and hypodermal cell layers (Correia et al., 2018).

The skin (exocarp) of the sweet cherry fruit consists of a cuticular membrane and several dermal cell layers which forms the epidermis (single layer of collenchyma cells) and the hypodermis (up to 7 cell of collenchyma subepidermal layers) having an insignificant number of stomata and which are non-functional at fruit maturity (Peschel et al., 2003).

The cuticle. Minimization of water loss, water proofing, protection against biotic and abiotic factors, fruit appearance, and textural properties are just some of the attributes of the cuticle.

The cuticular membrane is synthesized by the epidermis of fruits and all above-ground plant organs, and must be seen as a lipidized cell wall region (Guzman et al., 2014) whose properties in terms of water permeability are the focus of most theories regarding cherries cracking susceptibility.

The basic element of the sweet cherry cuticular membrane is cutin (whose synthesis is done exclusively by epidermal cells), a series of esterified fatty acids C16:0 or C18:1 (reported mainly for sweet cherries), synthesized in the plastids, to which a series of waxes (epicuticular and intracuticular waxes with triterpenoid acids-ursolic acid- as the main component) are added (Lara et al., 2015).

In phase of rapid increase in fruit mass (stage III of fruit development), the mass of the cuticular membrane and wax per unit fruit surface area decreases, this is due not only to the fact that fruit surface increased by about 311% (while e.g. mass of the cuticular membrane increased by 50% in this phase), but also resulted from changes in the compositional structure of the two elements (Figure 1), which may affect some wetting characteristics and mechanical properties of the cuticular membrane (Peschel S. et al., 2007).

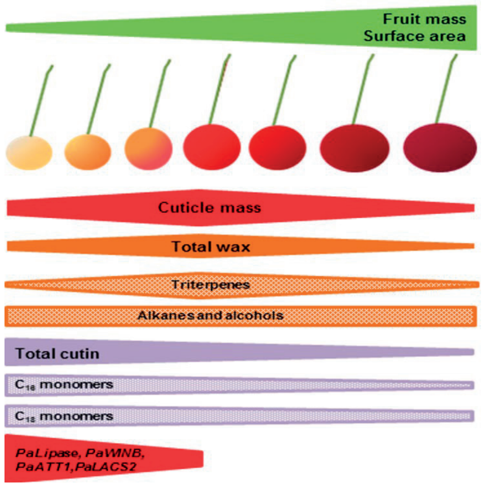

Figure 1. Changes in cuticle deposition and composition during the development of sweet cherry (Lara I. et al., 2015)

CURRENT STRATEGIES IN SWEET CHERRY FRUIT CRACKING MANAGEMENT

Gibberellic acid, a chemical compounds, that act as plant regulators, naturally synthesized in the plant, from the family of phytohormones (which can be both regulators and retarders or inhibitors of various plant traits or physiological processes), was most often associated with the idea of increasing plant resistance to biotic and abiotic factors, promotes growth and elongation of cells (Rothwell and Pochubay, 2014) increase fruit size and firmness, delays peel senescence, theoretically making it more difficult for fruit flies to infest the fruit (Birke et al., 2006), decrease mechanical injury and delay maturation for the late-maturing genotypes (Proebsting et al., 1973; Looney and Lidster, 1980) and delay exocarp coloration.

The signals regarding its use as a main pawn in the fight against fruit skin cracking have been mostly contradictory, due to a very high heterogeneity in terms of environmental conditions (Rothwell and Pochubay showed that the optimum is above 20°C), application doses, genetic diversity, application time and sometimes it depends on the use of surfactants (Rothwell and Pochubay, 2014).

Some studies showed that the application of gibberellic acid (GA_3) in the mandarin orange cultivar 'Nova' increases fruit splitting when applied at flowering, but reduces it when applied a little later, shortly after the end of the June drop (García-Luis et al., 1994). The results of the experiments initiated by Cline and Trought also showed in the same direction. They concluded that GA_3 sprays had positive effects on sweet cherry fruit quality parameters (weight, moisture content, firmness, soluble solids), but very unfavorable in terms of fruit cracking (amount of fruit splitting and size cracks) exceptionally wet weather at harvest (Cline and Trought, 2011). Completely different were the results obtained by Yildirim and Koyuncu (2010), who, in addition to an improvement in some parameters related to quality, their study showed that the GA_3 applications (straw colour) at the rate of 20 ppm decreased the cracking index in the fruit.

In terms of achieving satisfactory results on cherry fruit, most users agree that the timing of gibberellic acid application is crucial. This can vary from the end of the pit hardening phase, before the rapid period of fruit cell enlargement to straw colour, but always in the morning or evening, on windless days.

Facteau et al. (1989) suggest that if dose is increased, the result is reduction on flowering of sweet cherry ('Bing' and 'Lambert' cultivars in their experiment) for the next year, especially on 1-year-old wood. The reduction of transpiration on the surface of the fruit is the reason for using gibberellin, but in case of prolonged rains, the application of gibberellin can have opposite effects (Kaiser et al., 2019).

The latest innovations involve the use of **biofilms** (Figure 2) with a role in cuticle supplementation. Developed and patented by Oregon State University, the biofilm Parka (Cultiva, Las Vegas, NV) is a wax-based product, made from food grade, elastic and hydrophobic biopolymers, applied to supplement the fruit's surface cuticle and allow for increased elasticity and reduced cracking (Vance and Strik, 2018).

Although the product guarantees a reduction in the incidence of fruit cracking, not all experiments carried out with this product have shown this meaning (Vance and Strik, 2018).

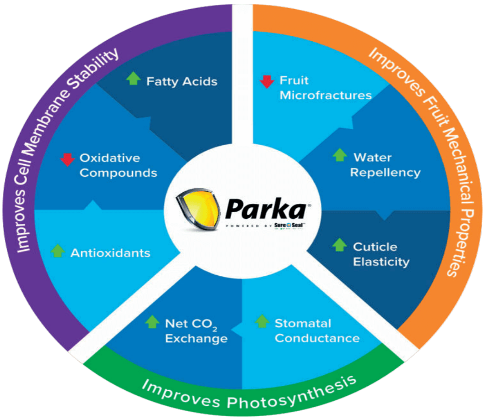

Figure 2. New biofilm with a role in cuticle supplementation, Parka (source https://www.cultiva.com)

Calcium physiology. As the fruit develops, the xylem (the only pathway through which calcium is transported) breaks the connection with the fruit, and the fruit's needs will be provided by the phloem, which leads to a continuous decrease of calcium in the fruit (Brüggenwirth et al., 2016).

Even if the results are contradictory, the use of calcium-based foliar products is used as a method to reduce sweet cherry fruit cracking.

Regarding the rationale for using calcium salts, it is based on two mechanisms: calcium salts are responsible for decreasing the osmotic potential for water uptake, and a decrease in the swelling of the cell wall simultaneously with the increase in adhesion between cells. The change in the osmotic potential following the application of calcium salts, some studies (at least at the theoretical level) have shown that it would be quite small (a reduction in the absorption of water through the skin with values between 8.3 and 16.2%) and which would be rather less visible under field conditions (Knoche et al., 2014; Moing et al., 2004). Schumann et al. (2022) demonstrated the effectiveness of Ca salts in reducing the swelling of cell walls (under laboratory conditions, by incubating epidermal segments in $CaCl_2$), the only impediment being the ability of these salts to penetrate through the intact cuticle, which implies their application immediately after rainfall when they will enter in contact with the emerging cracks.

In addition, Winkler and Knoche (2021) showed that the absorption of calcium is proportionally influenced by temperature and humidity on the surface of the skin, the pedicel cavity being the most important way for the calcium salts penetration into the fruit.

Genetics. As early as 1985, Cuartero et al. showed that fruit cracking has a strong genetic determinism involving several genes, and this character can be easily transmitted in the offspring. Encouraged by the good results obtained in tomato by Capel et al. (2015), Quero-Garcia et al. (2021), in an extensive study carried out between 2008-2016 which involved the multi-year analysis of a population of cherry hybrids (*Prunus avium*) - 3 hybrid combinations resulting from the crossing of 4 genotypes, 2 known to be very sensitive to cracking ‘Lapins’ and ‘Garnet’ and 2 that showed reduced susceptibility - ‘Regina’ and ‘Fercer’ - managed to identify three stable quantitative trait loci (QTL) for each type of cracking (in the area of the peduncular cavity, pistil and on the faces the fruit), which confirms the complexity of this phenomenon. From the breeder's perspective, locating quantitative trait loci (QTL) with different agronomic traits is very important for the implementation of marker-assisted selection strategies. But, as the authors go on to show, despite using multiyear values and analyses, the smallest confidence intervals for the most significant quantitative trait loci (QTL) spanned 4-5 cM (centimorgans), meaning that at least 100 genes are present in these ranges, which affected a realistic search for functional candidate genes responsible for cracking tolerance/susceptibility.

And in cherry, as in other species, increasing the genetic base through the selection of genotypes resistant to cracking and their introduction into breeding programs, remains an important direction to follow. Analyzing more than 200 cherry genotypes in the collection of Nikita Botanical Gardens in Crimea, Gorina et al. (2021) identified four genotypes that showed a high tolerance to cracking (‘Znatnaya’, ‘Zagadka’, ‘Kutuzovka’, ‘Zemfira’) and which can be used in programs to improve this character in cherry. At SCDP Iași, Corneanu et al. (2021) showed that through the hybridizations carried out in cherry breeding works, the new varieties obtained showed a much lower rate of fruit cracking than the parents used. Thus the new varieties ‘Cătălina’ (6.0%), ‘Margonia’ (1.3%), ‘Maria’ (9.3%), ‘Andreiaş’ (5.5%) and ‘George’ (5.8%) showed superior resistance to face cracking comparing to the varieties ‘Van’, ‘Boambe de Cotnari’, ‘Stella’, or ‘Fromm’ used as parents (between 10.0% and 70.3%).

On the other hand, experiences with focus on rootstocks have shown that they also influence the susceptibility to fruit cracking. Some trials conducted in The Dalles, Oregon, determined that ‘Mahaleb’ is generally the most susceptible rootstock, followed by ‘Mazzard’,‘Gisela 5’,‘Gisela 6’,‘MaxMa 14’,‘Krymsk 5’, and ‘Krymsk 6’ (Long et al. 2021).

Covers. The reduction in tree vigor has made possible the increasing use of tree cover systems to control fruit cracking. The high marketable value of the sweet cherry fruit could offset the rather high costs involved in installing such systems. In conditions of high precipitation, under Voen covering system, high valuable marketable yield can reach 85% and only 53% without cover due to high precipitation (Rubauskis et al., 2013).

Taking into account some less favorable aspects related to their use - high costs of establishment and maintenance, additional labor for installing them, problems related to dormancy (Quero-García et al., 2017), the negative balance of some quality parameters (Blanke and Balmer, 2008), the use of these systems in areas with high vulnerability is absolutely necessary.

In order to avoid such problems, some practices have been imposed as necessary - cover installation after bloom time (for example in Norway) or, most of the times, much later, near the fruit ripening (the third stage of fruit development), various colors covers for early ripening, retractable covers, etc. The complexity of these systems has increased - from the well-known the three-wire design system to the much more efficient and versatile Voen system Figure 3).

Figure 3. Three-wire design system (left side) and new Voen system (right side)

CONCLUSIONS

The phenomenon of cherry fruit cracking strongly affects the commercial value of production in many areas of the world, especially in the recent years with an evident climate change environment. Understanding the mechanisms underlying this phenomenon and choosing the best agrotechnical decisions. These are highlighted as follows: proper choice of the area to set up the plantation, the most suitable varieties and rootstocks, a good irrigation and fertilization programme and application of phytosanitary treatments and other special measures - like protective covers, and mineral supplements in areas with high climate vulnerability. All of these decisively influence the quality of production and the health of the plantation and make the difference between a successful or uneconomic plantation.

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THE CONTROLLED POLLINATION BEHAVIOR OF SOME INDIGENOUS VARIETIES OF CHERRY IN THE CONDITIONS S.C.D.P. BANEASA BUCHAREST

Florentina DOBRINESCU, Lenuța Constantina CHIRA, Daniel NAGY, Ligia ION, Viorel Cătălin OLTENACU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: florentina_dobrinescu@yahoo.com

Abstract

With a total remaining area of approximately five thousand hectares, after the existence of approximately 6900 ha with cherries was shown in 2013, Romania continues to be a country with relatively good productivity in terms of obtaining cherries. The cherry tree germplasm fund is part of the plant biodiversity that has developed over time in nature in a spontaneous and controlled way and that is the basis for the permanent improvement of cultivated varieties. The activity of collecting and preserving biological material in organized collections has a long tradition. The importance of this biological material is substantial, especially when we talk about the interaction of these genotypes with quarantine viruses, especially Plum pox virus, considered the most devastating. The pollination scheme used under SCDP Baneasa Bucharest conditions included one pollinating variety, Boambe de Cotnari, and four controlled pollinated varieties, Daria, Izverna, Superb and Severin.

Key words: *pollen, hybridization, fruits, quality.*

INTRODUCTION

In the improvement of horticultural plants, the history of the development of knowledge and achievements in the field were specific to the level of knowledge and the concrete possibilities of each time.

In the Middle Ages, the Renaissance period marked the moment when the first systematic plant breeding works began, so this moment can be considered as a second stage in the development of plant breeding works.

Beforehand, new technologies and culture methods were developed, species were introduced, greenhouses were built, etc. (Sestraș, 2004).

On a global level, there is an intense and continuous concern for the genetic improvement of cherry varieties as the main way to improve agricultural performance, harvest quality, increase the commercial value of the fruit and the only possibility of increasing disease resistance, with implications in reducing the costs of production and the level of pollution of the agro-ecosystem (Research Institute and Development for Fruit Growing Pitesti-Maracineni).

The cherry is part of the family Rosaceae, subfamily Prunoideae, genus *Prunus* (many botanists use the genus name *Cerasus* for the two species). The genus Prunus includes approximately 150 species, some of which are very important in fruit growing (those cultivated) and in breeding works (Cimpoieș, 2002).

Prunus avium (wild or bird cherry) is the species from which most cherry varieties were formed; in the spontaneous flora it is found in South-Eastern Europe, the Caucasus, North Africa, Asia Minor, China and shows a pronounced polymorphism. The trees grow up to 20-25 m in height, form a pyramidal crown and are pretentious to heat. According to Buia (1956), cited by Ardelean (1986), in our country, within the species there are three varieties: Sylvestris, Juliana and Duracina.

Cousin. Sylvestris Kirschl. (wild cherry) is the one from which the varieties with small, black, bitter fruits, with soft pulp and early ripening, such as Early May and Early (Fruhesteder Mark) come.

Cousin. Juliana L. (Ispas cherries), gave birth to varieties with medium and large black fruits, with soft and juicy pulp, sweeter than wild

cherries, with early and medium ripening. Ramon Oliva and French Timpurie are part of this variety.
Cousin. Duracina L. (stone cherries) is the variety from which the varieties with late ripening, large fruits, of different colors, yellow, red or black, with hard pulp (Bigarreau, "stone" varieties) come. This variety includes the most valuable cherry varieties grown in the world, including some well-known in our country: Germersdorf (synonymous with Schneiders's Späte), Hedelfinger, Napoleon (synonymous with Royal Ann), Dönissen and others (Cimpoies, 2002).
From the multitude of these species, the most valuable germplasm fund and sources of genes suitable for cherry breeding work are made up of local and improved varieties, belonging to the cultivated species. The variability of *P. avium* varieties presents important characteristics for culture and improvement, such as productivity, fruit quality, resistance to diseases, frost, drought, etc.

MATERIALS AND METHODS

The efficient use of genetic resources is the most important step in the activity of germplasm identification, collection, evaluation and preservation. The more genetically diverse the germplasm pool, the greater the possibilities of obtaining new hybrid varieties with increased productivity, superior quality, with resistance to adverse environmental factors and the action of phytopathogenic agents. The basic principle of the efficient use of plant genetic resources is for the breeder to constantly know the gene pool as well as the objectives he pursues in the process of creating new forms of biological material.
If an effective selection within the germplasm forms is desired then a pronounced variability of the material is required. This can be natural, but it can also be created by breeders if the natural variability does not meet the requirements of an enhanced diversity. Regardless of the method of creating new forms of biological material (varieties, hybrids), it is mandatory to know the direction of work and the objectives pursued. But even if these problems are very clear, it will never be possible to obtain valuable material if the germplasm pool is not sufficiently known and if its variability is not sufficient.
For these reasons, before starting any breeding process, a very clear evaluation of the initial breeding material, separately for each species, and the creation of databases that can be used by the breeder in order to facilitate his activities, are required.
The studies were carried out on a number of 5 varieties of cherry regarding the germinative capacity of the pollen and on a number of 4 varieties regarding the induced or controlled pollination. The germinative capacity of the pollen was determined according to the standard methodology.
The 5 cherry varieties were: Boambe de Cotnari, Daria, Izverna, Superb and Severin.

RESULTS AND DISCUSSIONS

Germinative capacity of pollen, fertility (self-fertility or self-sterility) and the quality of good or poor pollinator for other genotypes are particularly important characteristics in fruit tree species, with direct implications on the quantity and quality of the fruit harvest.
Even if the characteristics are determined genetically, they are also influenced by other factors, among which the climatic conditions, especially the temperature has a determining role.

Climatic conditions

The average temperature of January 2022 ranged from -11.5°C to 3.4°C. The highest values, over 2°C, were recorded in the center and south of Muntenia, including in the Bucharest area, at the Baneasa meteorological station. The deviation of the average January 2022 air temperature from the median of the standard reference interval (1991-2020) was positive over most of the country. Values above 3°C were recorded in the center and south of Muntenia. Analyzing the severity classes of thermal anomalies from January 2022, it can be seen that the thermal regime was extremely hot in the center of Muntenia.
The average temperature of February 2022 ranged from -9.7°C to 5.6°C. The highest values, above 4°C, were recorded in large areas of Muntenia. The deviation of the February 2022 mean air temperature from the median of

the standard reference interval (1991-2020) was positive. Large deviations, over 3°C, were recorded in Muntenia, including the Bucharest area.

The average temperature of March 2022 ranged from -11.8°C to 5.5°C. The highest values, above 4°C, were recorded in most of Oltenia, Banat and Crișana and locally, in the center and north-east of Muntenia. The deviation of the average air temperature in March 2022 from the median of the standard reference interval (1991-2020) was negative in all regions of the country. Deviation values higher than 1.5°C were recorded in extensive areas of the country. Analyzing the severity classes of the thermal anomalies from March 2022, it can be seen that the thermal regime was cold in most of Romania's territory. In southern Muntenia, it was very cold and extremely cold.

The average temperature of April 2022 ranged from -5.0°C to 12.7°C. The highest values, over 12°C, were recorded in areas in the east and south of Muntenia. The deviation of the April 2022 mean air temperature from the median of the standard reference interval (1991-2020) was negative over most of the country. Positive deviations had values below 1°C. In Muntenia, the thermal regime was warm.

The national average temperature of May 2022 was between 1.2°C and 19.5°C. The highest values, over 18 °C, were recorded in the south and northeast of Muntenia, but also locally in Banat. The deviation of the average air temperature in May 2022 from the median of the standard reference interval (1991-2020) was positive in almost the whole country. Analyzing the severity classes of the thermal anomalies from May 2022, it can be seen that the thermal regime was warm in most of Banat, Crișana, Oltenia and Muntenia.

The cherry requires a certain number of cold hours to complete the morphogenesis and microsporogenesis processes, so it can be assumed that in the climatic conditions of the January-May 2022 period, many cherry varieties did not accumulate the necessary cold, which affected the processes of pollen formation and pollination compatibility.

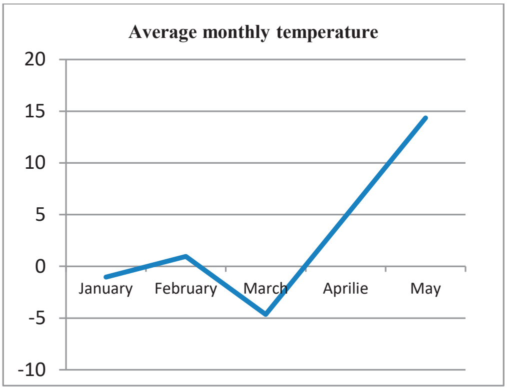

Figure 1. Evolution of the average air temperature between January and May 2022 in the Baneasa area of Bucharest

The germination capacity of the pollen

The data from Table 1 regarding the pollen germination capacity of the 5 varieties of cherry show values between 42.94% (Severin) and 68.75% (Boambe de Cotnari).

Table 1. Germination capacity of pollen in cherry varieties

Nr. crt.	Varieties	Microscopic field	Pollen grains		Germination percentage
			All	Germinate	
1	Daria	I	16	7	51.87
		II	14	7	
		III	17	9	
		IV	23	14	
2	Izverna	I	17	11	53.84
		II	15	8	
		III	19	9	
		IV	8	4	
3	Superb	I	11	6	50.45
		II	12	6	
		III	13	5	
		IV	17	10	
4	Severin	I	15	9	42.94
		II	12	5	
		III	14	6	
		IV	11	3	
5	Boambe de Cotnari	I	14	10	68.75
		II	15	9	
		III	13	10	
		IV	9	6	

It can be considered that the varieties with higher than average pollen germination percentage (Boambe de Cotnari, Izverna) were not influenced by the variable temperatures. The other genotypes, Daria, Superb and especially Severin, recorded anomalies in the process of pollen formation.

Controlled pollination

From the 5 genotypes, the Boambe de Cotnari variety was chosen as the pollinator variety.

The pollination scheme implemented was the following:

Combination I

(minimum 300 castrated and pollinated flowers)

Daria ♀ X Boambe de Cotnari ♂

Combination II

(minimum 300 castrated and pollinated flowers)

Izverna ♀ X Boambe de Cotnari ♂

Combination III

(minimum 300 castrated and pollinated flowers)

Superb ♀ X Boambe de Cotnari ♂

Combination IV

(minimum 300 castrated and pollinated flowers)

Severin ♀ X Boambe de Cotnari ♂

Table 2. The situation of fruit formation following controlled pollination

Nr. crt.	Varieties	Number of pollinated flowers	Number of fruits formed	Fruit binding percentage
1.	Daria	315	217	68.88
2.	Izverna	320	237	74.10
3.	Superb	310	194	62.58
4.	Severin	310	187	60.32
TOTAL		1255	835	66.53

From the data in Table 2, it appears that the total number of fruits formed was 835, which represents 66.53% of the total number of controlled pollinated flowers.

The highest number of tied fruits was recorded for the Izverna variety (237), and the Severin variety (187) had the lowest value.

The number of tied fruits compared to the 4 varieties was 208.75, according to the data in Table 3. The largest percentage difference compared to the average was registered by the Izverna variety (28.25%), and the smallest percentage difference, it was recorded in the Daria variety (3.95%).

Table 3. The difference recorded by varieties, in absolute and relative values, compared to the average number of fruits

Nr. crt.	Type	Number of fruits formed	Difference from the average (number)	Difference from the average (%)
1	Average varieties	208.75	-	-
2	Daria	217.00	+8.25	3.95
3	Izverna	237.00	+28.25	13.53
4	Superb	194.00	-14.75	7.07
5	Severin	187.00	-21.75	10.42

Figure 2. Detail of the cherry fruit branch

Figure 3. Controlled pollination

Figure 4. Daria variety detail

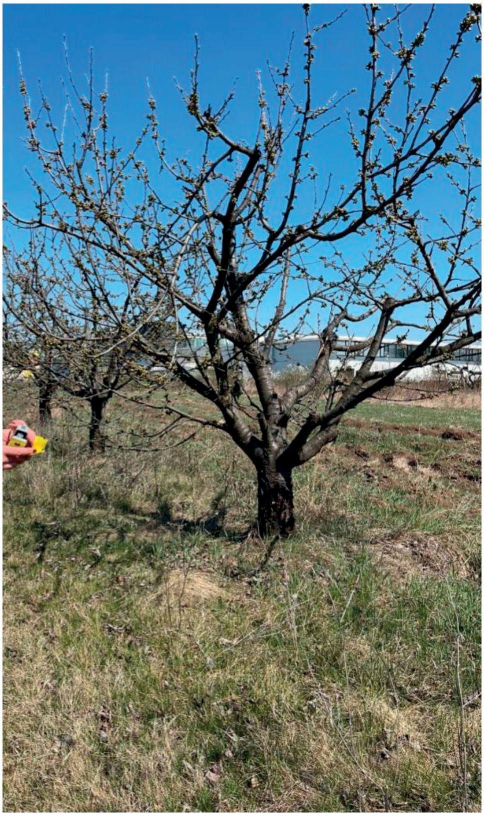

Figure 5. Aspect from the orchard S.C.D.P. Baneasa

Figure 6. Checking the binding of the fruits

CONCLUSIONS

The data regarding the germinative capacity of the pollen and the controlled pollination of a number of native cherry varieties, from the Orchard Baneasa Research and Development Station in Bucharest, in the climatic conditions from January to May 2022, show the normal variability of the absolute and average values, the characteristic genotypes of the cherry species.

There are, however, varieties that, by the values of the monitored parameters, are below the average of the analyzed genotypes. They are varieties more vulnerable to the high temperatures of the winter-spring period, which deregulate the organogenesis processes of the flower buds.

The pollen germination capacity of the 5 cherry varieties shows values between 42.94% (Severin) and 68.75% (Boambe de Cotnari).

The varieties Boambe de Cotnari and Izverna were not influenced by the variable temperatures during the study period.

The Severin variety registered anomalies in the process of pollen formation.

The number of fruits formed, after controlled pollination, represents 66.53% of the total number of flowers. The highest number of tied fruits was recorded for the Izverna variety (237), and the lowest value was recorded for the Severin variety (187).

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THE EFFECT OF FERTILIZERS ON THE QUALITY OF APPLE FRUITS

Ana Maria DODOCIOIU, Gilda-Diana BUZATU

University of Craiova, Faculty of Horticulture, A.I. Cuza Street, no. 13, Craiova, Romania

Corresponding author email: diana_buzatu@yahoo.com

Abstract

In the period 2019-2021, the effect of different doses of complex fertilizers of the 16:16:16 type applied alone and together with some foliar fertilizers of the Fertil Star and Aurora types on the production, quality and accumulation of some nutrients in the leaves of the 'Florina' apple variety was monitored, cultivated on a cambic faeziom soil type from Mehedinți. The productions were obviously marked by the complex application of the 3 macroelements, being between 20.35 t/ha and 25.61 t/ha. A clear influence of fertilization on the total dry matter content was found, between 11-17%, the increase being directly proportional to the intake of nutrients from the applied fertilizers. High concentrations of K cause their acidity to increase, which has the consequence of changing the ratio of sugar/organic acids and finally changing the taste in the sense of increasing the flavor. Nutrient elements in the leaves changed favorably as a result of the application of doses and types of fertilizer.

Key words*: apple, fertilizers, quality.*

INTRODUCTION

Apple (*Malus domestica* Borkh.) occupied third place as the most consumed fruits, worldwide (FAOSTAT, 2021). Five apple varieties were among the most popular for growing - 'Golden Delicious', 'Red Delicious', 'Granny Smith', 'Florina' and 'Melrose' (Petkova et al., 2022).

The 'Florina' apple variety has a French origin and it was imported in 1977 and now is quite common (Dobrevska et al., 2022).

Fresh apple consumption in the EU is approximately 15 kg per capita and is expected to increase by 1 kg by 2030, while consumption of processed apples is around 8 kg per capita (EC, 2020). Fruits and vegetables are the most important natural sources of vitamin C. Since apples are very popular globally, they could be an excellent source of vitamin C in the human diet (Lemmens et al., 2020).

Contemporary trends in horticulture are aimed at limiting the use of mineral fertilizers to the necessary minimum, which is to guarantee adequate profitability of production while maintaining high-quality fruit and at the same time preventing environmental pollution. In this situation, it is necessary to develop a rational method of N fertilization of apple orchards, allowing minimization of the use of fertilizers (reduction of costs) and reduction of the risk of contamination of the soil with an excess of nitrates, while achieving optimal tree growth, yield and fruits quality (Kowalczyk et al., 2022). Intensive apple production has primarily focused on increasing productivity through intensification of fertilizers and water, resulting in high production, financial and environmental costs (Stefanelli et al., 2010).

Potassium is among essential mineral nutrients with long-known importance for plant development and yields. Its optimal supply is very important under the conditions of climate change and in view of the need to obtain good yields of high-quality fruits (Kuzin & Solovchenko, 2021). Reaching this goal requires up-to-date fertilization management systems presuming balanced use of both organic and chemical fertilizers (Gitea et al., 2019). An adequate supply of K is essential for uptake of other nutrients and plays an important role in the formation of yield improvement (Xu et al., 2020).

Titratable acidity of fruit is an important parameter in determining fruit maturity and key determinant of fruit taste. It also serves as food substance and need by body in little amounts. (Azher et al., 2020). Researchers showed that titratable acidity of different apple varieties grown in world ranged from 0.10% to 0.36% respectively Chakespari et al., 2010, Vieira et al., 2009, and Durrani et al., 2010, to 0.60%, Dzanagov et al., 2021.

Increasing the acidity value of the fruits has the effect of changing the ratio of sugar/organic acids and finally changing the taste in the sense of increasing the flavor of the fruits (Dilmaghani et al., 2005).

In order to elucidate some aspects regarding the effectiveness of foliar and root fertilizers in an appple orchard, we conducted research in the period 2019-2021 in Mehedinți County with complex root fertilizers as well as with two foliar fertilizers.

MATERIALS AND METHODS

The experiment was located on a cambic faeziom soil type in Mehedinti County, in an apple orchard, with the 'Florina' variety comprising the following variants:

V1 - unfertilized control;
V2 - Fertil Star foliar fertilizer;
V3 - Aurora foliar fertilizer;
V4 - $N_{48}P_{48}K_{48}$ root fertilizer;
V5 - $N_{80}P_{80}K_{80}$ root fertilizer;
V6 - $N_{48}P_{48}K_{48}$+Fertil Star 5 l/ha;
V7 - $N_{48}P_{48}K_{48}$ +Aurora 4 l/ha;
V8 - $N_{80}P_{80}K_{80}$ +Fertil Star 5 l/ha;
V9 - $N_{80}P_{80}K_{80}$ + Aurora 4 l/ha.

Mehedinti County (44.63°N 22.88°E) is located in the southwestern part of Romania, on the left bank of the Danube.

Fertilizers were tested alone or in paired combinations, in comparison to an unfertilized control.

Applied foliar fertilizers have the following chemical composition:

Fertil Star: N-67%, P_2O_5-6%, K_2O-4.2%, Cu-0.025%, Fe-0.100%, Mn-0.010%, Zn-0.008%, B-0.020%, Mo-0.001%, chelates with EDTA Na

Aurora: N-10%, P_2O_5-0.5%, K_2O-3.5%, B-1.5mg/l, Ca-1g/l, Co-1.16 mg/l, Cu-3.8 g/l; Mn-0.08, Fe-1.4 g/l; S-3.8 g/l, Mg-1.8 g/l; Zn-1.5 g/l, biostimulant plant extract.

Complex extraradicular fertilizers 16-16-16, were applied in two stages: 1/3 of the dose in autumn and 2/3 of the dose in spring in the budding phase, the complex being incorporated into the soil at both stages.

Foliar fertilizers were applied in three stages:
- after tying the fruit;
- two weeks after the first foliar fertilization;
- two weeks after the second foliar fertilization.

For characterization of climatic elements were used information from the nearest weather station, namely Drobeta Turnu-Severin Weather Station. According to this data, the climate is Cfax type, continental climate, with a weak Mediterranean influence, with an average annual temperature of 10.8°C and an average annual rainfall of 539.9 mm.

The soil on which the experiment was placed was cambic faeziom type with the following characteristics:
- profile formed by horizons: Am, Bv, B/C, C;
- clay texture;
- medium bulk density (1.38-1.49 g/cm^3);
- medium total porosity (44.6-48.3%);
- hygroscopicity coefficient 4.76%;
- medium supply degree with nitrogen (3.26% humus, Nt = 0.14-0.15%);
- good supply degree with phosphorus (52 ppm P);
- very good supply degree with potassium (210 ppm K);
- weakly acid-neutral reaction (6.38-7.53);
- second quality class.

The 'Florina' variety is characterized by medium to large fruit size, with a smooth, greenish-yellow surface, the skin is hard, greenish-yellow, covered with red-orange with darker streaks on almost 80% of the surface (Petkova et al., 2020). The pulp is yellowish-white, with greenish shades, juicy, consistent, with a slightly acidic sweet taste.

Analytical methods were applied, as fallows. Total soluble dry matter content was determined using a digital refractometer at 20°C and expressed as %.

The titratable acid content (acidity) was determined by titration with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as indicator.

Redox titration determined vitamin C using Iodine solution of juice sample and finally determine the titrate required for standard (Mohammed & Hazim, 2016).

Statistical analysis were processed using the statistical package SPSS 17.

RESULTS AND DISCUSSIONS

The average production obtained in the three years of experimentation was between 20.35 and 25.61 t/ha. Radicular fertilization leads to higher yields depending on the applied dose: 22.4-23.55 t/ha. Unilateral fertilization with Fertil Star results in a small increase in production compared to the unfertilized control, 1.10 t /ha, which is only significant.

Very significant production increases are obtained by using higher doses of root fertilizer, respectively $N_{80}P_{80}K_{80}$ - 23.55 t/ha, as well as for complex fertilizers $N_{48}P_{48}K_{48}$, $N_{80}P_{80}K_{80}$ + foliar fertilizer Fertil Star - 24.83 t/ha and Aurora 25.61 t/ha (Table 1), which is in concordance with the results obtained by Zheng et al., 2016 and Dzanagov et al., 2021.

An important criterion in assessing the reaction of an apple tree to the application of mineral fertilizers is the fruit quality indicators (Doroshenko, 2006).

Table 1. Productions (t/ha) obtained for the Florina variety as a result of the use of different doses of root and foliar fertilizers on average for 3 years of experimentation (2019-2021)

Variant	Production	% to the unfertilized control	Difference from unfertilized control t/ha	Statistical significance
Unfertilized control	20.35	-	1.10	-
Fertil Star	21.45	105.2	1.75	x
Aurora	22.1	108.6	2.05	x
$N_{48}P_{48}K_{48}$	22.4	110.0	3.20	x
$N_{80}P_{80}K_{80}$	23.55	115.7	3.60	xxx
$N_{48}P_{48}K_{48}$ +Fertil Star	24.01	117.9	3.66	xxx
$N_{48}P_{48}K_{48}$ +Aurora	24.50	120.4	4.15	xxx
$N_{80}P_{80}K_{80}$ +Fertil Star	24.83	122.0	4.48	Xxx
$N_{80}P_{80}K_{80}$ + Aurora	25.61	125.8	5.26	Xxx

DL5% = 1.04 DL1% = 2.46 DL0.1% = 3.11

Note:
- No statistical difference compared to control;
- x - significantly positive compared to control;
- xxx - very distinctly significantly positive compared to control.

The production quality was assessed by the dry matter content (%), soluble dry matter (%), titratable acidity. These indicators are of particular importance in obtaining high quality fruit by balancing these indicators.

It can be clearly seen that by using different doses of root and foliar mineral fertilizers the total dry matter content increases.

Thus, for the unfertilized control, dry matter content has a value of 13.83%, while applying the two single foliar fertilizers, dry matter content increases to values of 14.59% and 14.92%, i.e. by 5.5 and 7.8%.

Table 2. Total dry matter content over the 3 years of experimentation depending on the dose of fertilizer

Variant	Dry matter %	% to the unfertilized control	Difference from unfertilized control t/ha	Statistical significance
Unfertilized control	13.83	-	-	-
Fertil Star	14.59	105.5	0.76	-
Aurora	14.92	107.8	1.09	xx
$N_{48}P_{48}K_{48}$	14.72	106.4	0.89	-
$N_{80}P_{80}K_{80}$	15.59	112.7	1.76	xx
$N_{48}P_{48}K_{48}$ +Fertil Star	15.09	109.1	1.26	x
$N_{48}P_{48}K_{48}$ +Aurora	15.25	110.3	1.42	xx
$N_{80}P_{80}K_{80}$ +Fertil Star	15.73	113.7	1.90	xxx
$N_{80}P_{80}K_{80}$ + Aurora	15.88	114.8	2.05	xxx

DL5%=0.9 DL1%=1.31 DL0.1%=1.77

Note:
- No statistical difference compared to control;
- x - significantly positive compared to control
- xx - distinctly significantly positive compared to control;
- xxx - very distinctly significantly positive compared to control.

The application of root mineral fertilizers has the effect of increasing the dry matter content to the values of 14.72% and 15.59%, i.e. by 6.4-12.7%. Similar values regarding dry matter content were also reported by Mitre et al., 2009.

The combined application of root and foliar fertilizers leads to significant or distinctly significant increases when using a dose of less than 48 kg of active substance (a.s.) and a significant increase when using the dose of 80 kg a.s./ha, the increase of dry matter content reaching values of 1.9-2.05%.

The soluble dry matter content (%) was also favorably influenced by the doses and types of fertilizers used (Table 3).

The soluble dry matter content has been slightly modified by the doses and types of fertilizers. This content, as can be seen from the data in Table 3, increases with the application of different doses and types of fertilizers, but the increases are less significant or even insignificant.

Thus, following the application of Fertil Star and Aurora foliar fertilizers alone, as well as with small doses of root mineral fertilizers 48 kg a.s./ha, the increases are insignificant (0.11-0.56%).

Only in the situation of fertilization with high doses of root mineral fertilizers 80 kg a.s./ha the percentage obtained increases, 0.64% and 0.88%, being significant or distinctly significant.

Table 3. Influence of fertilizers on soluble dry matter content, %

Variant	Soluble dry matter %	% to the unfertilized control	Difference from unfertilized control t/ha	Statistical significance
Unfertilized control	10.96	-	-	-
Fertil Star	11.07	101.0	0.11	-
Aurora	11.25	102.6	0.29	-
$N_{48}P_{48}K_{48}$	11.37	103.7	0.41	-
$N_{80}P_{80}K_{80}$	11.58	105.6	0.62	X
$N_{48}P_{48}K_{48}$ +Fertil Star	11.43	104.3	0.47	-
$N_{48}P_{48}K_{48}$ +Aurora	11.52	105.1	0.56	-
$N_{80}P_{80}K_{80}$ +Fertil Star	11.60	105.8	0.64	X
$N_{80}P_{80}K_{80}$ + Aurora	11.84	108.1	0.88	Xx

DL 5% = 0.61 DL1% = 0.80 DL0.1% = 1.01

Note:
- No statistical difference compared to control;
- X - significantly positive compared to control;
- xx - distinctly significantly positive compared to control.

The titratable acidity of apple fruits is expressed %, the determining factor of its value being the potassium concentrations that directly proportionally influence this quality indicator.

It can be observed from the data in Table 4 that for the unfertilized variant was registred the lowest titratable acidity value, 0.352%. Root fertilization with $N_{48}P_{48}K_{48}$ causes an increase of this indicator by 0.036% or by 10.2%. Root fertilization with $N_{80}P_{80}K_{80}$ and together with Fertil Star causes the increase of this indicator by 19.6% and, respectively 19.8%, compared to the unfertilized control.

The most obvious statistically guaranteed percentage increases are obtained in the case of using root fertilizer together with foliar fertilizers 18.2-27.8%.

Table 4. Titratable acidity according to different doses and types of fertilizers

Variant	TA %	% to the unfertilized control	Difference from unfertilized control t/ha	Statistical signify cance
Unfertilized control	0.352	100	-	-
Fertil Star	0.391	111.1	0.039	x
Aurora	0.402	114.2	0.050	xx
$N_{48}P_{48}K_{48}$	0.388	110.2	0.036	-
$N_{80}P_{80}K_{80}$	0.421	119.6	0.069	xxx
$N_{48}P_{48}K_{48}$+ Fertil Star	0.416	118.2	0.064	xxx
$N_{48}P_{48}K_{48}$+ Aurora	0.431	122.4	0.079	xxx
$N_{80}P_{80}K_{80}$+Fertil Star	0.422	119.8	0.070	xxx
$N_{80}P_{80}K_{80}$+ Aurora	0.450	127.8	0.098	xxx

DL5% = 0.037 DL1% = 0.041 DL0.1% = 0.056

Note:
- No statistical difference compared to control;
- x - significantly positive compared to control;
- xx - distinctly significantly positive compared to control;
- xxx - very distinctly significantly positive compared to control.

The content of vitamin C for the variety 'Florina' varied under the influence of fertilization between 8.1 mg/100 g and 8.9 mg/100 g in comparison with the control variant, for which the content of vitamin C recorded the value of 7.8 mg/100 g.

Table 5. The content of vitamin C in the 'Florina' variety

Variant	Vitamin C (mg/100 g)
Unfertilized control	7.8
Fertil Star	8.1
Aurora	8.3
$N_{48}P_{48}K_{48}$	8.5
$N_{80}P_{80}K_{80}$	8.9
$N_{48}P_{48}K_{48}$ +Fertil Star	8.2
$N_{48}P_{48}K_{48}$ +Aurora	8.5
$N_{80}P_{80}K_{80}$ +Fertil Star	8.4
$N_{80}P_{80}K_{80}$ + Aurora	8.8

The data correlate with those obtained by Dzanagov et al., 2021, for the same variety. Reporting to the unfertilized control where the recorded vitamin C content was 7.8 mg/100 g, the recorded increases were between 0.3-1.1 mg / 100 g.

It is observed that the highest amount of vitamin C is registered in the fertilized variants. The highest amount of vitamin C was recorded in the $N_{80}P_{80}K_{80}$ variant of 8.9 mg/100 g. In all the other fertilized variants, smaller but higher amounts of vitamin C are obtained compared to the unfertilized control.

According to Schuphan, 1956, Mapson, 1970, and Fisher, 1999, cited by Câmpeanu et al., 2009, apple contains 2-30 mg ascorbic acid per 100 g, depending on the cultivars.

CONCLUSIONS

The experience with different doses of root and foliar fertilizers carried out during 2019-2021 on the apple crop, 'Florina' variety, highlighted the following aspects:

- Both the foliar fertilizers used, Fertil Star and Aurora, as well as the complex root fertilizers in doses of 48 and 80 kg a.s./ha increased production by 5.2-8.6% for foliar fertilizers and by 10.0-15.7% to root fertilizers, registering significant increases.
- The application of foliar fertilizers in combination with root fertilizers had the effect of obtaining significant production increases of 17.9-25.8%, greater increases being obtained when Aurora foliar fertilizer was used, which has a more complex chemical composition as well as biostimulatory extract from plants. In this case, production reaches 25.61 t/ha compared to 20.35 t/ha obtained for the unfertilized control.
- Analyzing some production quality indicators such as total dry matter, soluble dry matter, titratable acidity and vitamin C, it was found that these indicators were also improved especially as a result of the combined use of foliar fertilizers with root fertilizers, when very significant increases of these indicators are obtained.

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STUDY REGARDING THE BEHAVIOR OF THREE CHOKEBERRY CULTIVARS (*ARONIA MELANOCARPA*) CULTIVATED IN ORGANIC SYSTEM

Damian DRAGOMIR[1, 2], Dorel HOZA[1], Viorel-Cătălin OLTENACU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Fruit Research and Development Station Băneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

Corresponding author email: dragomir.damian@scdpbaneasa.ro

Abstract

The paper presents data related to the evolution during 2021-2022, of three chokeberry Aronia varieties, grown organically in the farm belonging to FRDS Băneasa, located in N-E of Bucharest in Afumaţi, Ilfov County, in the Vlasiei Plain, a subunit of the Roman Plain. Aronia melanocarpa, is a crop that issuitable for organic system, and the importance of fruits is given by the fact that it has special nutraceutical qualities, mainly due to the high contain of anthocyanins and flavonoids among other important substances that can reduce the risk of serious illness. The study was continued on a plantation established in 2020. The chokeberry varieties under observations were: 'Melrom', 'Nero' and 'Galicjanka'. Planting distances 1.5 m/3 m, in 2 variants, plants canopy conducted as bush. In 2022, number of shoots and inflorescences per plant, shoots length and the number of fruits per inflorescence were counted. Also, fruit weight, size, sugar content, pH and citric acid for each variety was determined and compared to the 2021 results. The study will continue in order to gather more data for the organic cropping system of Aronia.

Key words: *chokeberry, organic technology, cultivars, canopy,growth dynamic.*

INTRODUCTION

Black chokeberry [*Aronia melanocarpa* (Michx.) Elliott] is a deciduous shrub native to North America, botanically classified as belonging to the family Rosaceae. Shrub of chokeberry grows 1.2 to 2.4 m producing beautiful white flowers and navy-blue berries of tart-sweet taste and aromatic flavour (Jeppsson, 2000; Brand, 2010). It is possible to establish a chokeberry plantation in any climate. Because it is a cold-hardy plant tolerant of very low temperatures even below –35°C and it is not sensitive to the spring frosts (Bussières et al., 2008). Chokeberries are noted for their modest requirements and adaptability to different soil types (sandy, acid and humid soils) and soil management systems.

According Ioan Viorel Raţi (2016) increased population demands for healthy food has led to the expansion of shrubs cultures by: the emergence of new growing areas; new systems of cultivation (crop protected in tunnels, greenhouses, growing without soil, organic production).

Moreover, only a limited number of global cultivars are well-suited to the specific pedoclimatic conditions present in Romania, as indicated by Ligia Ion (2007, the National Program for Rural Development 2014-2020). The traditional agricultural practices in Romania emphasize the use of clean technologies, allowing for the establishment of environmentally friendly farming zones. This commitment to eco-friendly approaches is reinforced by the increasing demand for organic products, transforming organic farming into a viable source of income for the rural population, as highlighted by many authors Sumedrea D. et al. (2014) and Rati I. V. (2008).

The paper reports on the research done in an organic system between 2021 and 2022 on three cultivars of *Aronia melanocarpa*: 'Galicjanka', 'Melrom', and 'Nero'. The chokeberry and leaves of *Aronia melanocarpa* provide a multitude of health benefits because of the presence and high concentration of different bioactive components, including vitamins, minerals, and polyphenolic compounds (Szopa et al., 2017). Organic farming systems have become increasingly interested in cultivating aronia because of its high demand for cultivation due to its nutritional value and resistance to both biotic and abiotic factors.

MATERIALS AND METHODS

The study took place over a span of two years, from 2021 to 2022, at the Fruit Research and Development Station Băneasa, Moara Domnească Farm, situated in Ilfov County, Romania. The site is positioned approximately 25 kilometers northeast of Bucharest in Ilfov County. The locale features a continental temperate climate with clearly delineated seasons. Elevated temperatures define summers, while winters experience cold conditions, often accompanied by recurring droughts. The average yearly temperature is 14°C, and the annual precipitation falls within the range of 500 to 650 mm. The zenith of precipitation, frequently characterized by intensity, occurs between May and July. During winter, prevailing air circulation follows patterns from the East and North-East, transitioning to the West in the other months.

Maximum wind speeds vary between 11.5 and 20.5 km/h. The soil type is reddish luvisol. In the depressed areas and in the crevices there are reddish luvisols and stagnosols (Dragomir et al., 2002).

The organic Aronia plantation was established in the spring of 2020 (Figure 1.), and the experiment was designed with two experimental factors: cultivars: 'Melrom', 'Nero', 'Galicjanka', mulching of plant rows with two systems: 1. bare soil, 2. mulched with rests from wood. The mulch was of approx 20 cm thick.

Figure 1. Aronia plants newly planted in 2020 mulched

In 2020, the soil between the rows was regularly tilled and lacked grass. In contrast, by 2022, a consistent grass cover was present, as shown in Figure 2. The experimental design employed a randomized block design, with three replications and a total of sixty black chokeberry plants. During the trial, standard cultural, training, and pruning practices were used, including drip irrigation.

Figure 2. Aronia plants in organic experimental field mulched (2022 Farm Moara Domnească)

Variables linked to generative potential, such as the count of shoots and inflorescences per plant, along with the number of berries per cluster, were assessed through counting. Biometric measurements of the fruits were conducted using 60 specimens for each cultivar and variant. The height and diameter of the fruit were measured using an electronic caliper, while the weight of the fruit was determined using an electronic balance with precision up to 0.01 g. Sugar content expressed as % Brix, was determined by the electronic refractometer (Hanna instruments HI 96800), and the pH value was measured by pH Meter Hanna HI 700630.

The data gathering process produced a number of variants, and the MS Excel "Data analysis" addon was used to statistically evaluate. The Duncan-T-Student concordance test (t) with a probability of 0.05 was used to assess the samples for the statistical hypothesis on differences between variants since there were two variations in the study and data were gathered for each cultivar in 2021 and 2022. (Dragomir et al., 2002).

RESULTS AND DISCUSSIONS

There were no significant differences found between treatments or years in terms of generative potential, more especially in relation to the number of shoots per plant. In the year 2021, the cultivar 'Melrom' in V2 showed the most shoots per plant.

The application of wood chip mulch in both years has affected the average number of shoots per plant for all cultivars. More specifically, on V1, the mulched variant showed 14-13 shoots per plant in 2021, but on bare soil (V2) in 2022, there were 18-15 shoots per plant (Table 1)

Table 1. Comparison of the generative potential of aronia in 2021 versus 2022 regarding the number of shoots per plant

Treatmen t	Cultivar	Average no. shoots/plant 2021	Average no. shoots/plant 2022
V1 - mulched	GALICJANKA	11	12
	MELROM	14	14
	NERO	15	13
V2 - bare soil	GALICJANKA	18	14
	MELROM	19	15
	NERO	17	15

As far as the average number of flower clusters per plant is concerned, V2 had the highest count in 2021 - an average of 17.33 flowers. By contrast, V1 recorded 15.67 inflorescences on average per plant in the same time frame.
The variation on the average number of inflorescences per plant between the years 2021 and 2022 is notable. The mean number of inflorescences per plant in V1 and V2 in 2022 was 156 (min. 153 and max. 197 clusters/plant), as compared to 15.66, with a minimum of 10.33 and a maximum of 19.33 in 2021 (Figure 3).

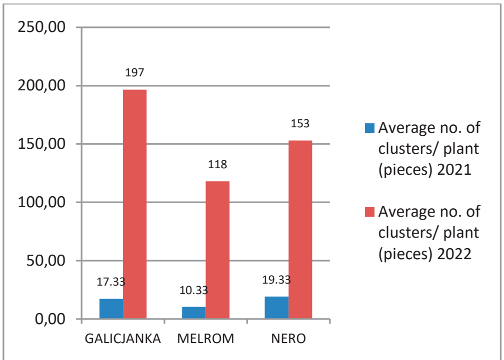

Figure 3. Average number inflorescences per plant 2021 compared with 2022

Number of fruits/cluster

Regarding the data on the number of berries per cluster, it here was little difference between the cultivars in the two years.
When comparing 2021 and 2022 with respect to the average number of fruits/cluster, the 'Galicjanka' cultivar in V2 had the highest average number of fruits per cluster (25.11 fruits/cluster in 2021) (Figure 4). The lowest average number of fruits/cluster has been observed by 'Nero' in V2 in 2021. If we compare the cultivars production of fruits/ cluster in 2022 the highest number is observed also at the cultivar 'Galicjanka' in V2 (23.00 fruits) and the smallest number of fruits per cluster by 'Galicjanka' in V1 (16.00 fruits).

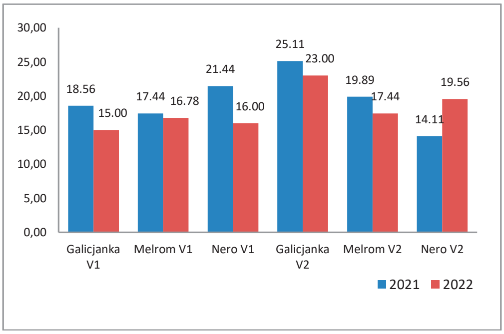

Figure 4. Average number of fruits/cluster 2021 compared with 2022

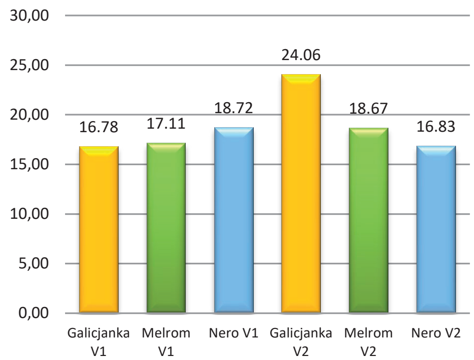

Figure 5. Number of fruits/cluster average for 2021 and 2022

In the Figure 5, it is shown the overall production of fruits per cluster expressed as an average number for each cultivar observed in 2021 and 2022. 'Nero' in V2 showed an exceptionally high average number of fruits per cluster - 16.83 berries per cluster - apart from the 'Galicjanka' cultivar with the highest average fruits/cluster of 24.06.

Fruit analysis

Different sizes of chokeberry fruit cultivars were noted based on the results (Table 2).

The ‘Galicjanka’ cultivar had the largest fruit weight in 2021, followed by fruits from the ‘Nero’ and ‘Melrom’ cultivars. The average weight of ‘Galicjanka’ fruit in 2021 was 1.09 g, whereas in 2022 it was 0.71 g in V1 and 0.52 g in V2. The average weight of the other two cultivars, Melrom and Nero, is comparatively lower at 0.55 g.
Jeppsson (2000b; 2000a) classified large fruits as weighing 71 g for 100 berries; this value is lower than the weight of 109 g resulting from extrapolation of the weight of the ‘Galcjanka’ fruit to 100 fruits. Since the average fruit weight recorded during our experiment corresponds with the first crop year, the conclusion of (Jeppsson, 2000), these cultivars ranged in weight from 65 to nearly 95 g. Every relevant detail about the minimum and maximum fruit sizes for the years 2021 and 2022, as well as the average fruit weight, is included in Table 2.
Fruit pH in 2022 was determined to be at normal levels; the highest value was found for ‘Nero’ fruits in V1 (3.46), closely followed by ‘Melrom’ (4.45) and ‘Galcjanka’ (3.40) in V1. Based on a probability test with a 0.05 P (T<=t) probability, there is no statistically significant difference between the pH cultivar values. Many fruit characteristics as well as the amount of sugar in the Aronia fruit all influence its quality. The flavor profile of aronia fruit is often described as having a complex combination of dryness, tartness, earthy undertones, and astringency. The elevated tannin content of the fruit generates a profile reminiscent of characteristics found in dry red wine. Aronia fruit typically needs a significant sugar content in order to be considered edible.
From the Figure 6, it can be observed that maximum average value of Brix % in 2022 has been reached by 'Melrom' V2 (19.10) followed by 'Galicjanka' V1 with (18.80) Brix units.

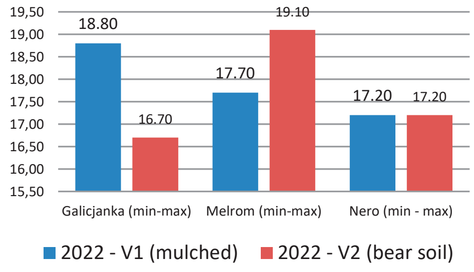

Figure 6. Minimum and maximum, sugar content of aronia fruits (harvest 2022)

In addition to the fruit's sugar content, the citric acid content was measured in 2021 and 2022. This particular compound is particularly significant because it is essential to the fruit's overall composition and influences both its flavour and possible health benefits. It has been noted from the laboratory results that in 2022.

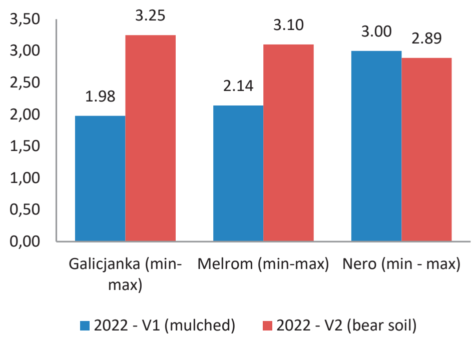

Figure 7. Minimum and maximum citric acid content of aronia fruits (harvest 2022)

The highest acidity value was found in 'Galicjanka' V2 (3.25), and simultaneously in V1 (1.98) had the lowest level (Figure 7.).
As can be seen from Table 2 values were calculated for each year, and all three cultivars were exhibiting comparable results.

Table 2. General characteristics of aronia fruit in 2021 and 2022

Fruit characteristics	Cultivars		
	‘Galicjanka’	‘Galicjanka’	‘Nero’
Average weight of fruit (g) V1 best results			
2021	1.09a	1.03a	1.08a
2022	0,71a	0.55a	0.51a
Fruit size diameter (mm) min.-max.			
2021	9.55-14.40	9.45-14.61	9.66-14.35
2022	10.40-13.09	10.81-13.45	10.00-13.22
Mean fruit size			
2021	11.98a	12.03a	12.01a

2022	11.81a	11,83a	11.38a
Sugar content (% Brix) Mean (% Brix)			
2021	18.70a	16.90a	18.35a
2022	18.80a	17.70a	17.20a
pH mean			
2021 (august harvest)	4.01a	3.98a	4.03a
2022 (july harvest)	2.62a	2.62a	2.95a

Considering that the study was conducted over two years after the plantation was started in 2020 and that there were notable changes in the weather (particularly with regard to rainfall and solar insolation) during this period, the results showed that, on average, the year 2021 produced better fruit than the year 2022 in all categories related to its quality and physical attributes.

CONCLUSIONS

The objective of the study was to investigate changes from 2021 to 2022 in the organic aronia plantation and the characteristics of its cultivars. But drawing firm conclusions is difficult because of the significant variations in the weather throughout these two years. Early findings from 2021 and 2022 suggest that *Aronia melanocarpa* is a very adaptable plant, demonstrating the ability to adapt to the soil and climate in Romania's southeast.

The cultivar 'Galicjanka' had the greatest results in both research years, followed by 'Melrom' and 'Nero'.

It is evident that the V1 mulched form has yielded superior outcomes when comparing the two varieties, V1 with mulch and V2 with bare soil. This can be attributed to its capacity to hold onto water, guaranteeing the plants a consistent supply of moisture when needed.

To collect further data from years with similar climatic circumstances, variables influencing plant growth and development, and the expression of distinctive features in aronia cultivars, more study is required.

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DEPENDENCIES BETWEEN VEGETATIVE AND REPRODUCTIVE PERFORMANCES IN 'SHOPSKA ALENA' RASPBERRY CULTIVAR

Diyan GEORGIEV, Maria GEORGIEVA, Denitsa HRISTOVA

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture Troyan, 281 Vasil Levski Str., Troyan, Bulgaria

Corresponding author email: d.georgiev.slujeben@gmail.com

Abstract

The scientific experiment was conducted during the period of 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The object of the study is the '*Shopska Alena' raspberry cultivar. The plantation was created in the autumn of 2016. Correlation analyzes were made between the vegetative indicators: number, height and thickness of the shoots with the yield. The highest values for height (1.38 m) and thickness (8.41 mm) of the shoots were recorded in the second year of the experiment, and the highest yield was obtained in the third year - 1.69 kg/1m^2. A high correlation dependence between the height and thickness of the shoots was recorded through all three years and between the height and thickness with the yield in the second and third year.*

Key words: *cultivars, fruits, raspberries, reproductive indicators, vegetative indicators.*

INTRODUCTION

In the last twenty years, Europe has occupied the largest share in rasperry production (72.5%), followed by America (25.4%) and Asia (1.9%).

World production has grown by 21.9% in the last 5 years. According to FAO, the main European raspberry producers are: the Russian Federation (153 827 t), Serbia (93644 t) and Poland (85616 t). (https://www.fao.org/faostat/en/?#data/QCL/visualize).

Raspberry takes the first place among the small-sized fruit species in Bulgaria. Under the new economic conditions, there is an increase in occupied production areas in Bulgaria (except 2020 and 2021), due to the appropriate agroecological conditions for its cultivation, increased prices for producers and the rapid return of the insurance funds invested.

In recent decades, the demand for small-sized fruit, including raspberries, has increased many times because of their optimal yields, healthy ingredients and the ability to obtain attractive food with rich biochemism and high market value (Ivanova et al., 2012).

The cultivar structure in Bulgaria is made up of Bulgarian and introduced raspberry cultivars. Most of them are high-yielding, large-fruited, resistant to abiotic and biotic stress factors, with a good opportunity for mechanized harvesting and transportation (Boycheva, 1999; Boycheva, 2001; Georgiev et al., 2012; Domozetova et al., 2014; Bozhanska et al., 2019; Serbezova et al., 2019).

Profitability of raspberry fruit production is determined by the correct choice of cultivar, suitable agroclimatic conditions of the habitat (temperature, wind, precipitation, pests) and modern cultivation technology (Woznicki et al., 2016; Contreras et al., 2019).

The aim of the present research is to study the correlational dependences between the vegetative and reproductive indicators of 'Shopska Alena' raspberry cultivar, grown under the agro-climatic conditions of the Troyan region.

MATERIALS AND METHODS

The scientific experiment was conducted in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The planting scheme is 3.00/0.50 m, as the experiment was laid out in three replications, each one linear meter of the intra-row area of the plantation.

The plantation is located on a north-eastern slope, at an altitude of 460 m.

The soil on which the plantation was made is a gray forest, typical of the Troyan region.

Its mechanical composition is heavy sandy-clay, moderately eroded with a low content of humus (Atanasova, 2021).

The raspberry cultivar 'Shopska Alena' was created in 1954 by crossing [(*Prussia x England*) x *Newburgh*] and was recognized by the State Cultivar Comission in 1970 (Popov and Hristov, 1970). The shrubs are fast-growing, forming a large number of medium thick shoots. The cultivar tolerates temporary heat and droughts well during the harvesting period. Fruits are average-sized to large-sized with a spherical shape. It begins to ripen towards the end of June, the beginning of July. It is characterized by great fruitfullness. The cultivar has great potential for yield and high fruit quality (Hristov et al., 1988; Boycheva et al., 1994; Serbezova, 2019).

The following biological and economic indicators were monitored:

- average number of shoots per linear meter;
- average height of shoots (m);
- average thickness of shoots (mm), measured at 10 cm from the soil surface;
- average fruit weight (g);
- average yield 1/m^2 (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 regarding the vegetative indicator average number of shoots report that there is no significant variation in the values during the three-year period, which were in the range of 12.33-13.33 number (Table 1). The average for the period was 12.66 number, which shows that the cultivar has good shoot formation. The average plant height was greater in the first (1.35 m) and second year (1.38 m). The average shoot height of 1.24 m was recorded for the period. The average thickness of the shoots was significantly higher in the second year (8.41 mm), as the lowest was recorded in the third year (6.64 mm). The average thickness for the period was 7.48 mm, which necessitated the use of a supporting structure for growing the plants. According to Jenning (1980), "thicker shoots are more favourable to fruiting branches".

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

Average number of shoots 1/m^2	Average height of shoots (m)	Average shoots thickness (mm)	Yield (kg/1 m^2)	Average fruit weight (g)
2018				
12.33	1.35	7.40	0.51	3.26
2019				
12.33	1.38	8.41	0.90	2.23
2020				
13.33	0.98	6.64	1.69	2.50
Average for the period 2018-2020				
12.66	1.24	7.48	1.03	2.66

The lowest average yield was obtained in the first experimental year (0.51 kg/1 m^2), whereas the highest value was registered in the third year (1.69 kg/1 m^2). The average yield for the period was 1.03 kg/1 m^2. The largest fruits were gathered in the first experimental year (3.26 g). The average fruit weight for the period was 2.66 g, which defines it as medium-large. The fruit weight is correlated with the height and diameter of the fruit, the size of the receptacle, the number of fruiting bodies and the seed weight, as it increases during the ripening process and decreases during pre-ripening (Serbezova, 2019). According to Kichina (1990) no definite correlation was found between the size and shape of the raspberry fruits.

A very high correlation dependence was reported in 2018, from the correlation analyzes, between height and shoot thickness indicators (0.93) (Table 2). It was high for number of plants and height (0.86) and high but with a negative sign between shoot thickness and yield (-0.88). A significant correlation was reported between number of plants with plant thickness (0.60) and negative for shoot height and yield (-0.64).

Table 2. Correlation dependences between vegetative and reproductive indicators in 'Shopska Alena' in 2018

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	0.86	1		
Thickness	0.60	0.93	1	
Yield	-0.15	-0.64	-0.88	1

In the following year 2019, the correlation analyzes showed a very high correlation between shoot height and yield (0.98) and between plant thickness and yield (0.92) and a high correlation between height and plant thickness (0.82) (Table 3).

Table 3. Correlational dependences between vegetative and reproductive indicators in 'Shopska Alena' raspberry variety in 2019

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	0.09	1		
Thickness	-0.50	0.82	1	
Yield	-0.10	0.98	0.92	1

A significant but negative correlation was reported between shoot number and thickness (-0.50). The obtained results regarding the correlation dependencies differ to a significant extent compared to the previous year.

In the third experimental year, very high correlation dependences were registered between the indicators number and height of shoots (0.99), number and thickness of the plants (1.00) and height with the thickness of the shoots (0.99) (Table 4). High correlations were reported between plant thickness with yield (0.87), number of shoots with yield (0.88) and plant height with yield (0.81). Based on the correlation dependences between the indicators, a repetitiveness in the three years of high to very high correlation of the height with the thickness of the shoots can be reported.

Table 4. Correlational dependences between vegetative and reproductive indicators in 'Shopska Alena' raspberry cultivars in 2020

	Number of shoots	Height	Thickness	Yield
Number of shoots	1			
Height	0.99	1		
Thickness	1.00	0.99	1	
Yield	0.88	0.81	0.87	1

CONCLUSIONS

The present study analyzed the interconnections between the vegetative and reproductive indicators of the Bulgarian raspberry cultivar 'Shopska Alena', grown under the soil and climatic conditions of the Troyan region.

During the three-year period, the 'Shopska Alena' cultivar had good shoot formation, medium-thick shoots, which necessitated growing them on a support structure. A high to very high correlation dependence between shoot height and thickness was reported over the three-year period. Repetitiveness of results from high to very high correlation was recorded for number with plant height, shoot height and thickness with yield.

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THE INFLUENCE OF DIFFERENT ROOTSTOCKS AND PLANTING SYSTEMS ON SEVERAL APRICOT CULTIVARS' GROWTH IN THE BUCHAREST AREA

Imad Jabbar Wadi AL SUWAID[1], Ana Cornelia BUTCARU[2], Cosmin Alexandru MIHAI[1], Florin STĂNICĂ[1, 2]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Centre for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author emails: mcosminalexandru@yahoo.com, ana.butcaru@qlab.usamv.ro

Abstract

This study presents the growth and fruiting parameters of some new apricot varieties cultivated in Bucharest. The research was conducted on 28 apricot cultivars (Prunus armeniaca L.) in the Experimental Field of the Faculty of Horticulture, USAMV of Bucharest. Myrobalan 29C (M29C), Saint Julien A (SJA), and Grande Ferrade 677 (GF677) rootstocks were used, with two different planting systems, Parallel-U and Trident. The biometric parameters included trunk cross-sectional area (SST), axis cross-sectional area (SSB), canopy volume, and the total number and length of annual shoots under their influence. Generally, the cultivars grafted on M29C rootstock increase more vigor than those grafted on GF677 and Saint Julien A, regardless of the system planting. The use of the Parallel-U system compared to Trident involved the development of the SSB, with higher values in some studied cultivars. Depending on the purpose of growing the plants and the climatic conditions of the cultivation area, it is good to use rootstocks and the corresponding cultivation system to obtain the best performance of the cultivars used.

Key words: *Trident, Parallel-U, Saint Julien A, Myrobalan 29C, GF677.*

INTRODUCTION

Apricot, scientifically known as *Prunus armeniaca* L., belongs to the Rosaceae family group of diploid trees. The origin of this plant is Central Asia (Hormaza, 2002; Janick, 2005). Globally, the apricot cultivation area was nearly 560,000 hectares, which led to the harvest of about 4 million tons per year (FAO, 2022).

Pruning is one of the most critical technical functions in the cultivation technology of fruit trees. During the plant growth period, proper pruning produces strong branches with a suitable angle in the plant; this issue ensures that maximum light reaches the plant canopy and increases the strength and durability of its components. Balanced conditions are provided for the growth and development of flower and fruit buds in the tree. In addition, proper pruning in fruit trees causes the regeneration of damaged branches and controls the size of the tree canopy and the plant height (Demirtas et al., 2010).

Pruning in temperate regions is generally performed at the end of winter and the beginning of spring at the same time as the plant's dormant season (Demirtas et al., 2010). Proper pruning in the plant optimizes the relationship between shoot growth, available leaf surface, and photosynthesis, which ultimately affects the production of high-quality products (Lang, 2001).

Various planting systems, such as Vertical axis, Bi-Baum, Trident, etc., are effective in orchard management. Increasing planting density in orchards is also one of the results (Lauri & Lespinasse, 2000; Robinson et al., 2011; Hoying et al., 2012). Increasing the number of axes to two in the form of Bi-Baum, Tatura trellis, Parallel Y, etc.; also, increasing the number of axes to three in the Trident systems and even expanding the number of axes to four in Mikado led to the development of new canopy shapes in trees (Widmer & Krebs, 2001; Musacchi, 2008; Dorigoni et al., 2011; Stănică & Platon, 2011; Elkins & DeJong, 2011).

In Mediterranean regions, in apricot cultivation, Myrobalan rootstock and trees obtained from wild apricot are commonly used (Ercisli, 2009; Miodragovic et al., 2019). Rootstock affects scion phenology, vegetative growth, fruit quality, plant performance, and tolerance to biotic stress and nematodes (Layne, 1994). The regression relationship between tree height, canopy diameter, and branch height was linear. Moreover, it was said that there is a positive and non-linear relationship between trunk cross-section and tree height, canopy diameter, branch length, and canopy volume.

Due to the limited compatibility between scions and rootstocks originating from different climatic conditions, studying and evaluating the climatic compatibility of different apricot rootstocks is vital and essential (Bujdoso et al., 2019). In each cultural system, depending on the climatic conditions, the right combination of rootstocks and scions should be determined and selected in research programs (Opriță & Gavat, 2018).

In the study carried out in Romania, it was also found that the use of a Trident planting system in the apricot culture led to the facilitation and improvement of the productivity, stability, and efficiency of the new varieties introduced in Romania (Stănică & Eremia, 2014).

Our study presents the growth and fruiting parameters of some new apricot varieties cultivated in Bucharest.

MATERIALS AND METHODS

The research was conducted on 28 apricot cultivars (*Prunus armeniaca* L.) in the Experimental Field of the Faculty of Horticulture in Bucharest. Myrobalan 29C (M29C), Saint Julien A (SJA), and Grande Ferrade 677 (GF677) rootstocks were used, with two different planting systems, Parallel-U and Trident. The monitored biometric parameters included trunk cross-sectional area (SST), axis cross-sectional area (SSB), canopy volume, and the total number and length of annual shoots under their influence.

The research period was 2020-2022.

The plantation was established in 2017. Two rows containing 33 trees per row had planting distances of 4 x 2 m, with tall concrete posts and four wires used to support the canopy. In the Bi-Baum system, the third row had 45 trees with a 4.5 x 1.5 m planting distance. The shape of the canopy resulted from manual pruning carried out annually in the summer in two stages, the first being before the appearance of the fruits and the second after harvesting.

The biological material consisted of the newest apricot cultivars, tested in the specific Bucharest conditions. On the Trident system were cultivated Faralia/M29C, Fareli/SJA, Anegat/M29C, Farbali/M29C, Farbali/SJA, Farbela/M29C, Farclo/SJA, Farclo/M29C, Farlis/M29C, Fartoly/SJA, and Primaya/SJA. In the Bi-Baum (2.0 m) were cultivated Wonder Cot/M29C, Lady Cot/M29C, Delice/M29C, Lilly Cot/M29C, Milord/ M29C, Swired/M29C, Congat/M29C, Mikado/M29C, Lido/M29C, Med Flo/M29C, and Flopria/M29C. In the Bi-Baum (1.5 m) were cultivated Congat/GF677, Primando/ SJA, Primaya/SJA, Rubista/M29C, Portici/ M29C, Pisina/M29C, CMBU/M29C, Bergeron/M29C, Vitilo/M29C, Boccuccia Liscia/M29C.

The methods used to evaluate the growth and fruiting parameters of some new apricot varieties planted in the Bucharest area were:

The formula determined the **cross-sectional area of the trunk** (SST):

$SST = \pi \times r^2$,

where r = D/2, D = trunk diameter.

The trunk diameter was measured 30 cm above the grafting point using a digital caliper.

The formula determined the **cross-sectional area of the axis** (SSB):

$SSB = \pi \times r^2$,

where $r = P/2 \times \pi$, P = axis section perimeter.

The perimeter of the axis section was measured 30 cm from the starting point, and the measuring tape was used. They were calculated for the two or three axes and then averaged the values.

To determine **the volume of the canopy**, the formula was used:

$V = \pi \times 3^{-1} \times r^2 \times h$,

where:

r = the length of the canopy in the direction of the row and the width of the canopy at an angle of 90 degrees to the row (average of these two data/ 2 = r);

h= the tree's height from the first branches to the top (without the height of the trunk).

The number and length of fruiting branches were determined each year during the dormant period of each tree in the experiment.
Statistical analysis of data was performed with Microsoft Office (Excel) and SPSS programs, using a 5% probability level.

RESULTS AND DISCUSSIONS

The cross-sectional area of the trunk (SST)
In the series of cultivars with the Trident canopy shape, the highest value of SST in the first year of the study was observed in the cultivar Farbali/M29C (42.22 cm^2), and the lowest at Anegat/M29C (24.71 cm^2) and Farlis/M29C (22.80 cm^2). In the second year of the study, the highest SST value was observed in Farbali/M29C, and Faralia/M29C cultivars, and the lowest was recorded in Anegat/M29C. In the third year of the study, the highest values were recorded in the cultivars Farclo/M29C, Farbali/M29C, Farely/M29C, and Faralia/M29C (Table 1).

Table 1. Evolution of SST during the period 2020-2022 (cm^2) at the Trident system

Variant	2020	2021	2022
Anegat/M29C	24.71b	25.67b	30.72c
Faralia/M29C	32.71ab	41.66a	43.84abc
Farbali/M29C	42.22ab	42.39a	46.35ab
Farbali/SJA	33.94ab	33.95ab	39.52bc
Farbela/M29C	29.87ab	28.77ab	34.88bc
Farclo/M29C	37.29ab	38.62ab	52.69a
Farclo/SJA	28.60ab	31.61ab	38.45bc
Farely/SJA	36.42ab	38.72ab	45.91ab
Farlis/M29C	22.80b	30.33ab	33.30bc
Fartoly/SJA	29.44ab	33.25ab	36.59bc
Primaya/SJA	28.38ab	32.59ab	42.39abc
Average	31.49ab	34.33ab	40.42abc

Analyzing the influence of rootstock on SST growth, SJA induced a lower increase than M29C rootstock in Farbali and Farclo cultivars in all three years of study.
In the three years of research, the series of apricot cultivars in the Bi-Baum (2.0 m) planting system showed similar results. Thus, the highest reported increase in trunk cross-sectional area was in Lido/M29C and Med Flo/M29C cultivars compared to the other cultivars studied in each research year. Maximum values ranged between 25.90 cm^2 (2020), 29.48 cm^2 (2021) and 35.22 cm^2 (2022).

Table 2. Evolution of SST during the period 2020-2022 (cm^2) at the Bi-Baum (2.0 m) system (M29C rootstock)

Variant	2020	2021	2022
Congat	19.35ab	19.62abc	25.26abcd
Delice	21.79ab	24.46ab	26.95abcd
Flopria	12.86bc	15.00Bc	18.69cd
Lady Cot	19.28ab	20.75abc	25.41abcd
Lido	25.90a	29.49A	35.22a
Lilly Cot	18.91ab	22.42Ab	27.66abc
Medflo	24.01a	27.72A	34.39ab
Mikado	11.02bc	14.55Bc	20.35bcd
Milord	21.06ab	23.35Ab	28.09abc
Swired	8.24c	9.19C	13.32d
Wonder Cot	20.80ab	22.24Ab	23.59abcd
Average	18.14ab	20.41abc	24.88abcd

Swired/M29C showed the most diminutive increase relative to the SST parameter in each year of the study, 8.24 (2020) - 9.19 (2021) - 13.32 (2022) cm^2. Similar behavior was shown by Mikado/M29C and Flopria/M29C cultivars (Table 2).
In the series of apricot cultivars in the planting system Bi-Baum (1.5 m), Primando/SJA and Primaya/SJA showed the highest increases in SST over the three years of the study, and Bergeron/M29C and Rubista/M29C the lowest values (Table 3). The other cultivars had no significant differences between them.

Table 3. Evolution of SST during the period 2020-2022 (cm^2) at the Bi-Baum (1.5 m) system

Variant	2020	2021	2022
Bergeron/M29C	10.67b	12.90b	17.81c
Boccuccia Liscia/M29C	13.79ab	17.66Ab	19.82bc
CMBU/M29C	13.21b	17.38Ab	23.86bc
Congat/GF677	15.53ab	20.19a	22.49bc
Pisana/M29C	15.30ab	19.19Ab	21.46bc
Portici/M29C	14.36ab	19.29Ab	21.29ab
Primando/SJA	19.34a	22.76A	28.32a
Primaya/SJA	16.33ab	20.92a	35.02c
Rubista/M29C	13.09b	16.28ab	16.97bc
Vitillo/M29C	14.54ab	18.19ab	25.01bc
Average	14.39ab	18.26ab	23.00bc

Comparing the growth rate for SST in the Primaya/SJA variety between the two planting systems (Trident; 2.0 m) and (Bi-Baum; 1.5 m), it was observed the significantly higher values presented in the Trident system compared to the Bi-Baum (28.38/16.33 cm^2 - 2020; 34.83/20.92 cm^2 - 2021; 42.39/35.02 cm^2 - 2022).

Comparing the evolution of the Congat cultivar on the two rootstocks M29C and GF677 in the Bi-Baum planting system (19.35/15.53 cm^2 - 2020; 19.62/20.19 cm^2 - 2021; 25.26/22, 49 cm^2 - 2022) there was a more substantial increase in M29C in the first and last year of the study.

The cross-sectional area of the axis (SSB)

In analyzing this growth parameter, the average surface area of the axis section was taken into the analysis (two for Bi-Baum and three for Trident). In cultivars led with the Trident planting system, values ranged between 5.08 cm^2 (Farclo/SJA), 6.22 cm^2 (Farlis/M29C), and 11.38 cm^2 (Faralia/M29C) (similar value for Fartoly/SJA, Farclo/SJA, and Farclo/ M29C) in 2020; 5.52 cm^2 (Farclo/SJA) and 13.31 cm^2 (Fartoly/SJA) in 2021; 7.34 cm^2 (Farclo/SJA) and 16.23 cm^2 (Faralia/M29C) in 2022. M29C rootstock showed more vigorous growth than SJA in the three years studied in Farclo and Farlis cultivars.

In the series of cultivars Bi-Baum (2.0 m), cultivar Lido/M29C had the highest value of SSB in the three years studied, similar to cultivar Med Flo/M29C. Swired/M29C, Mikado/M29C, and Flopria/M29C showed the lowest increases each study year compared to the other variants. The values varied between 4.82 cm^2 and 12.44 cm^2 in 2020, 5.15 cm^2 and 13.57 cm^2 in 2021, 6.69 cm^2 and 16.46 cm^2 in 2022.

In the case of plants cultivated in the system Bi-Baum (1.5 m), SSB varied between 4.23 cm^2 (Rubista/M29C, similar to Bergeron/ M29C) and 8.42 cm^2 (Primando/SJA) in 2020; 5.57 cm^2 (Rubista/M29C, similar to Bergeron/M29C) and 11.30 cm^2 (Primaya/ SJA) in 2021; 5.53 cm^2 (Rubista/M29C) and 14.53 cm^2 (Primaya/SJA) in 2022.

Comparing the SSB values of the Primaya/SJA variant between the two Trident and Bi-Baum systems planting (8.77/7.44 cm^2 in 2020, 10.56/11.30 cm^2 in 2021, 14.18/14.53 cm^2 in 2022), similar values were observed in each year of the study.

Analyzing the influence of the two rootstocks M29C and GF677 on the SSB values of the Congat cultivar in the Bi-Baum planting system, 8.43/6.10 cm^2 in 2020, 9.14/7.95 cm^2 in 2021, 12.79/9.05 cm^2 in 2022, it was observed as in the case of SST, significantly higher increases induced by M29C rootstock compared to GF677.

Canopy volume study

In this study, the canopy volume was analyzed every year for each variant, even if the cultivation technology required annual shortening of the tree height by the needs of the space allocated to each plant, a fact that influenced the absolute values.

Following the application of the cultivation technology, in the series of varieties cultivated in the Trident system, the values varied between 1.91 m^3 (Fartoly/SJA) and 4.02 m^3 (Farbali/M29C) in 2020; 2.86 m^3 (Farely/SJA) and 3.83 m^3 (Farlis/M29C) in 2021; 3.61 m^3 (Farbali/SJA) and 5.99 m^3 (Primaya/SJA).

In the Bi-Baum (2.0 m) planting system, canopy volume values ranged between 1.48 m^3 (Flopria/M29C, similar to Mikado/M29C and Swired/M29C) and 2.95 m^3 (Lido/M29C) in 2020; 1.62 m^3 (Swired/M29C similar to Mikado/M29C and Flopria/M29C) and 3.35 m^3 (Milord/M29C, similar to Lilycot/M29C, Med Flo/M29C, Lido/M29C, and Wonder Cot/M29C) in 2021; 2.08 m^3 (Swired/M29C) and 4.98 m^3 (Med Flo/M29C).

The study of the response of the different variants in the Bi-Baum (1.5 m) planting system revealed a variation of tree canopy volume values between 1.96 m^3 (Portici/M29C) and 3.56 m^3 (Pisana/M29C) in 2020; 1.93 m^3 (Primando/SJA) and 4.10 m^3 (CMBU/M29C) in 2021; 1.88 m^3 (Portici/M29C) and 6.06 m^3 (Pisana/M29C) in 2022.

In the Primaya/SJA cultivar, the canopy volume in the two Trident and Bi-Baum systems varied between 2.33/2.76 m^3 in 2020, 3.74/2.95 m^3 in 2021, 5.99/5.33 m^3 in 2022.

The Congat cultivar, in the Bi-Baum system, was led on the two rootstocks M29C and GF677 at a canopy volume that varied between 1.97/2.21 m^3 in 2020, 2.76/2.55 m^3 in 2021; 3.85/2.73 m^3 in 2022.

Number of fruiting branches and annual growths

Being a more vigorous rootstock, M29C influenced the number of mixed and anticipated branches and total annual growth compared to SJA.

In the study of the different varieties, it was found that the variety Lido had the highest number of branches in both years of the study. Also, total annual growth and Congat cultivar had the lowest values (Bi-Baum, 2.0 m).

Comparison of the variants according to the dynamics of the SST and SSB

Comparing the resulting matrices with SST and SSB values in each variant, a similar development behavior was observed in some cultivars. Thus, in cultivars led in the Trident planting system (Figure 1), Anegat/M29C and Farlis/M29C showed similar growth behavior. Also, Farely/SJA and Primaya/SJA variants.

In the Bi-Baum (2.0 m) planting system, similarity in growth behavior was observed in Swired/M29C, Mikado/M29C, and Flopria/M29C; Milord/M29C and Lilycot/M29C; Lido/M29C and Med Flo/M29C (Figure 2).

In the Bi-Baum (1.5 m) planting system, similarities in growth behavior were observed in Portici/M29C, Pisana/M29C, and Congat/GF677; Rubista/M29C and Bergeron/M29C (Figure 3).

Tree growth, assessed by trunk cross-section area (Kumar et al., 2014), was influenced by rootstock and planting systems in this study. In this study, plants grafted on M29C in the Trident variants resulted in higher SST and SSB values than SJA. The effect of the rootstock on the growth stages has been confirmed (Beckman et al., 1992; Layne, 1994).

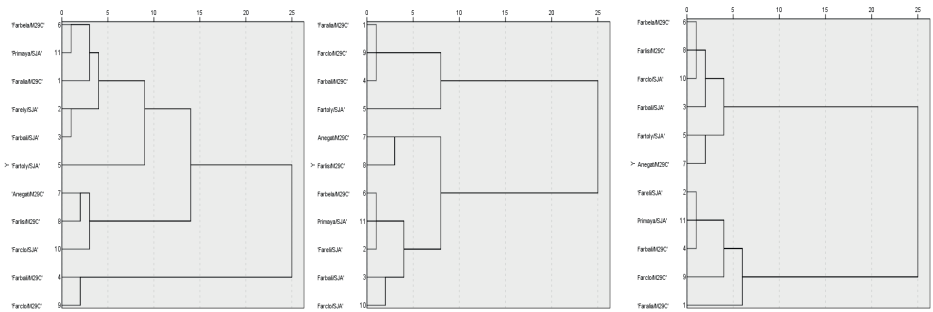

Figure 1. Variant clusters under the influence of SST and SSB in the Trident planting system (2020-2022)

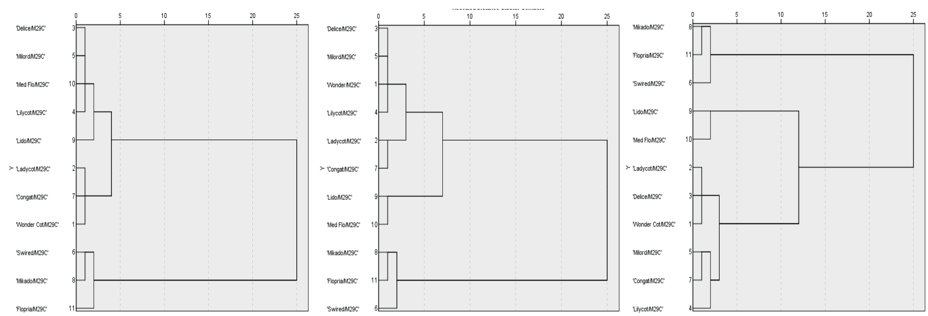

Figure 2. Variants cluster under the influence of SST and SSB in the Parallel-U, 2.0 m (2020-2022)

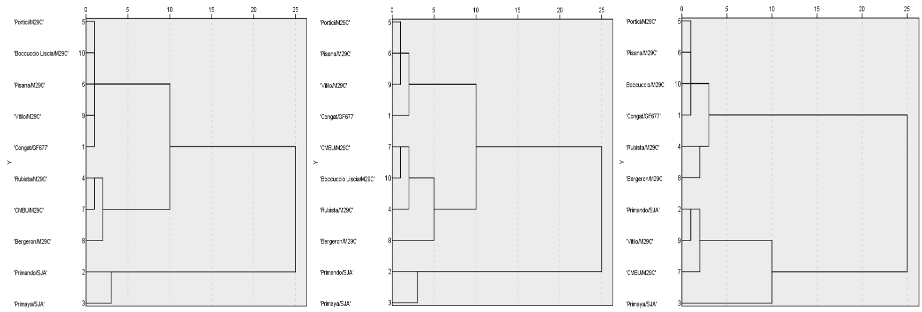

Figure 3. Variants clusters under the influence of SST and SSB in the Parallel-U, 1.5 m (2020-2022)

The choice of a suitable rootstock is one of the critical factors affecting orchard management. Plant growth, the quality of the fruits produced, and the plant's response to environmental conditions are affected by rootstocks. The effect of the rootstock on the plant also varies depending on the cultivar and the existing environmental conditions. It was explained that the rootstock influence on the growth parameters of the plant is stronger than the influence of the scion (Son & Küden, 2003; Egea et al., 2004; Sitarek & Bartosiewicz, 2011; Milošević et al., 2015; Milošević & Milošević, 2019; Pászti et al., 2022). According to the results of our research, the use of Myrobalan resulted in more significant increases in SST compared to St. Julien A, confirmed the Milošević & Milošević (2019) study results. The positive effect of Myrobalan on plant growth was also confirmed by Sosna & Licznar-Małańczuk, (2012) and Milošević et al. (2015).

In the planting system Bi-Baum (1.5 m), which used three rootstocks, M29C, SJA, and GF667, it was found that although SJA is known as a rootstock of medium vigor, in some variants, it caused growth higher compared to other rootstocks. This aspect is probably related to the shape of the canopy, Bi-Baum. The genetic code of the rootstock is efficient in plant growth. However, the different responses of the grafted variety in different rootstocks cannot be excluded (Hernández et al., 2010).

The trend to have higher densities in commercial apricot orchards has increased. Changing the number of vertical axes in each tree makes increasing or decreasing the cultivation density per unit area possible. In general, in commercial orchards, the presence of one or two axes is promoted (Dorigoni et al., 2011; Stănică, 2019).

Using the two planting systems, Bi-Baum and Trident, allows easier light penetration into the plant's canopy, the production structure is easily renewed, the performance increases, and the quality of the fruits is improved. In addition, maintenance of the plant is more accessible. Also, the tree's growth power is distributed on three or two vertical axes, creating an appropriate balance in the canopy structure (Stănică & Eremia, 2014). The Bi-Baum system is one of the most efficient when using rootstocks with medium-high vigor (Wertheim, 1998). One of the advantages of dividing the plant's growth power into 2 or 3 axes is the reduction of pruning time and the control of tree growth. Another advantage is the acceleration of canopy formation (Musacchi, 2008). The increase in SSB, SST, and the canopy volume in cultivars driven by this form can be related to the number of axes and branches in the plant, which leads to a better distribution of compounds resulting from photosynthesis.

CONCLUSIONS

According to the results of this study, it was found that using different rootstocks produces a different response in the plant in terms of vigor and growth speed. Different planting systems led to varying effects on the biometric parameters, influencing plant growth indicators. The research showed that the canopy volume was influenced by the two culture systems, Trident and Bi-Baum. Similarities of growth were highlighted in different cultivars throughout the study period.

Overall, the research showed that among the rootstocks used in different cultivars, M29C significantly affected branch production in the tree compared to SJA, confirming the effect of higher vigor on the trees—also, the Trident canopy form led to higher vegetative growth than Bi-Baum.

Depending on the purpose of growing the plants and the climatic conditions of the cultivation area, it is good to use rootstocks and the corresponding cultivation system to obtain the best performance of the cultivars used.

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CHILLING AND HEAT REQUIREMENTS OF TEMPERATE STONE FRUIT TREES (PEACH, NECTARINE, AND APRICOT)

Imad Jabbar Wadi AL SUWAID[1], Alexandru BUCUR[1], Ana Cornelia BUTCARU[2], Cosmin Alexandru MIHAI[1], Adrian ASĂNICĂ[1], Florin STĂNICĂ[1, 2]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Centre for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author emails: anabutcaru@gmail.com, mcosminalexandru@yahoo.com

Abstract

Like other temperate woody species, stone fruit trees (peach, nectarine, and apricot) must accumulate a specific amount of chilling during endodormancy and heat during eco dormancy for proper growth and development. Each cultivar has particular requirements, and special attention is needed to know them for an optimal decision support system. This paper aims to present the available information on the chilling- and heat requirements of peaches, nectarines, and apricots, focusing on the methods used for their determination. The results also reflected the necessity to standardize the methodology with the possibility of extending to new cultivars tested in different areas.

***Key words**: chilling hours; chilling units; chilling portions; growing degree hours.*

INTRODUCTION

Like other temperate woody species, peach, nectarine, and apricots must accumulate a specific number of chilling units during endodormancy and heat during eco dormancy for proper growth and development. Each cultivar has specific requirements, and special attention is needed to know them for an optimal decision support system.

This paper aims to present some available information on the chilling and heat requirements of peaches, nectarines, and apricots, focusing on the methods used for their determination. The reviewed data were organized into five sections: (1) General data; (2) Endo dormancy: chilling hours accumulation. Models and adjusted models; (3) Comparison between models justified by local climatic conditions; (4) When endo dormancy ends and starts heat accumulation (eco dormancy); (5) Heat accumulation models and patterns studied for different cultivars.

RESULTS AND DISCUSSIONS

(1) General data

Winter dormancy, the annual life stage of the tree between leaf drops and bud break, is the deciduous fruit tree mechanism to avoid damage from cold or freezing weather and has two stages that cannot be visually separated in the field.

In the first part, called endodormancy, tree growth is limited in the plant. A certain amount of cool temperature is required to end this first stage. This is referred to as the Chilling Requirement. Temperatures between -1°C-15.5°C contribute towards ending this first stage of winter dormancy, with temperatures between 1.6°C-10°C contributing the most chilling (Niederholzer, Scalabrelli & Couvillon, 1986).

In the second part of winter dormancy (eco dormancy), growth is controlled by an external factor - temperature. Each deciduous plant species requires a certain amount of heat to begin growing after the first stage of winter dormancy has been completed.

In conclusion, deciduous fruit trees need some cool weather and warm temperatures to start growing. Different tree species need different amounts of chilling and warm temperatures to bloom and grow (Niederholzer).

When the specific amounts of chill or heat are not completed, trees are affected by different disorders (Parker et al., 2019).

Chilling and heat requirement is critical in selecting cultivars for a given geographical region. Chilling requirements are generally largely satisfied in the temperate zone before the end of the cold season. Flowering happens too early, and low temperatures can induce an essential yield loss by frost.

Late blooming in stone fruit has been the objective of most breeding programs.

In subtropical areas, insufficient chilling is an economic problem. Symptoms of insufficient chilling are delay in flower and vegetative bud break, bud breaks spread over an extended period, or, in severe cases, a total lack of bud break.

Cross-pollination is needed in the field, so cross-compatible and simultaneously flowering cultivars must be selected as cultivars with similar chilling and heat requirements (Razavi, 2011).

Since the accumulation of temperature in the first 30 days after bloom plays a decisive role in fruit phenology, it has been found that increasing its temperature more than the specified temperature during spring after flowering in peach trees causes problems in the formation of fruit in these trees. Growing degree hours accumulate during the first 30 days after bloom reduces the number of days between the full bloom date and the reference date (Lopez & Dejong, 2007).

Investigating the growth pattern and phenological stages of early-maturing peach trees in the Mediterranean region showed that after 225 cold units to break dormancy, 6,244 growing degree hours (GDH) are required to reach full bloom, and 27,106 GDH are required before harvesting the fruit. About growing degree days (GDD), the heat requirement for full bloom was 329, and for fruit, harvesting was 1246 (Mounzer et al., 2008). It has been explained that by increasing the duration of the plant's exposure to chilling, the time and heat accumulation of breaking flower buds in peaches decreases exponentially (Okie & Blackburn, 2011). The study of peaches and nectarines cultivars found that the intensity of rest was lower in the flower buds. The resting intensity in the flower bud accompanied by chilling is reduced with a lower ratio than the leaf bud. In addition, it was found that the flower and leaf buds had different endo-dormancy depths but almost similar chilling requirements (Gariglio et al., 2006). It has been explained that terminal vegetative buds have a lower chilling requirement in the peach plant than lateral vegetative buds and flower buds. Meanwhile, the cold requirement in lateral vegetative buds and flower buds of the Redhaven cultivar was almost similar. In addition, it has been found that a temperature of 7.2°C is effective in meeting the cold requirement compared to 2°C or 3°C. Increasing the duration of chilling (2,040 hours) decreased the hours of growing degree required for the opening of vegetative buds regardless of temperature. However, the growing degree hours required for the flower buds were reduced by increasing the chilling duration to 7.2°C (Scalabrelli & Couvillon, 1986). The investigation has shown that the number of days to full bloom and the heat requirement for blooming peach trees negatively correlated with the accumulated chilling hours. In addition, it was found that the accumulated chilling hours had a significant negative correlation with the fruit shape index and fruit tip lengths. This issue confirms the effect of the number of chilling hours on the appearance of the fruit in dimensions and size. Hence, reducing the number of chilling hours during winter delays the bloom date in the tree and leads to the production of deformed fruits in peach. This issue can also be applied to other fruit trees in temperate regions (Yong et al., 2016).

Today, there are concerns about climate change and the lack of chilling requirements in trees with high chilling requirements (Parker & Abatzoglou, 2019). The study of 69 Japanese apricots found that the chill requirement in these cultivars ranged from 24 to 82 chill portions based on the dynamic model. The heat requirement in these cultivars was also observed between 691.9 and 2634.7 growing degree hours. The difference in the cold requirement of the studied cultivars makes it possible to select suitable cultivars for each region with specific climatic conditions (Zhuang et al., 2016).

(2) Endo dormancy: chilling hours accumulation. Models and adjusted models

The primary parameter in all the algorithms is the temperature. It can be measured by data loggers or specific sensors attached to meteo stations. Nowadays, there are many meteo data available worldwide on different platforms. https://www.ecad.eu/ presents an international meteo station with free daily data for 50-100 years in the past. ECA & D receives data from

85 participants from 65 countries, with 13 elements at 23,335 meteorological stations.

Other local meteo stations (on site) with free data or not are https://fruitsandnuts.ucdavis. edu/ chill-calculator, https://www.meteoblue.com, input companies (Syngenta, Bayer, etc.) and more.

Temperature can be recorded daily (Tmin., Tmax., Tavg.), hourly, 30 minutes, 15 minutes, etc. In the phenology algorithms, hourly temperature is used. If this is not available, there are more algorithms to transform daily data into hourly temperature (Chow & Levermore, 2007; Chițu, 2010).

The most used models to quantify the chilling hours are:

The chilling hours model (known before the 1950s) has been widely used for its simplicity and ease of use.

- Establishes that a cold hour (CH) corresponds to an hour with a temperature value between 0 and 7.2°C (this range of temperatures is considered to affect dormancy completion).
- Temperatures below 0°C do not affect slowed biological processes, and those above 7.2°C (45 F) were considered not low enough to affect dormancy completion.

Hutchins (1932), cited by Weinberger (1950); Richardson et al. (1975); Anderson et al. (1986).

The Utah model

- is based on the quantification of cold units (CU);
- establishes different temperature ranges with different contributions to dormancy completion;
- One cold unit corresponds to one hour for temperatures between 2.5-9.1°C, considered most effective in completing dormancy;
- Other temperature ranges have:
- 0.5 unity (1.5-2.4°C and 9.2-12.4°C),
- zero contribution (<1.4°C and 12.5-15.9°C) or
- negative (>16°C) at rest.

Richardson et al. (1974). There are more variations of the model, like the North Carolina model (Shaltout & Unrath, 1983; Anderson & Seeley, 1992), the Positive Utah model (Linsley-Noakes & Allan, 1994), Modified Utah Model (Linvill, 1990), in the effort to simulate the local conditions better.

The dynamic model (initiated in the 1980s):

- proposes accumulating an intermediate value according to low temperatures that can be reversed by higher temperatures (first stage).
- Once the value has reached a certain level, cold portions are added permanently, unaffected by higher temperatures.

Fishman et al. (1987), Luedeling (2012), https://ucanr.edu/sites/fruittree/How-to_Guides/ Dynamic_Model_-_Chill_Accumulation/ (University of California), Fadón et al. (2020), Pantelidis & Drogoudi (2023).

The importance is given to the sequence of temperatures during the cold season**.** Similar temperatures at different times of the season can affect chill accumulation differently.

(3) Comparison between models justified by local climatic conditions

Researchers applied the existing chilling accumulation models in different cultivation areas and compared the results with the field reality (Luedeling, 2012; https://fruitsandnuts. ucdavis.edu/chill-calculator).

Miranda et al. (2021) published “fruclimadapt: An R package for climate adaptation assessment of temperate fruit species”, describing the functions defined for chill hours(), chill_units(), chill_portions(), GDD_linear(), GDH_linear(), and GDH_asymcur().

Luedeling (2012) and most researchers compa-red at least two to three methods on specific cultivars for calibration. In the Lueding (2012) study, the Dynamic model was considered the best model for the species applied.

Zhu et al. (2022), in the study of flowering phenology in peaches and comparing different models with the purpose of monitoring flowering, found that the investigation of phenology based on BBCH comparison and the use of the random effect model in comparison with other models provides researchers with more accurate information. The highest score (98.82) correlated with the harmonic average of model accuracy and recall was obtained in this model. It is worth noting that real-time images recognize the specific color range and area, and the information related to the heat requirement improves the model's performance. This information facilitates the management of plant breeding, such as heat stress management, water requirements, and breeding programs.

The comparison of two critical data models and critical chilling models by Miranda et al. (2013) in Peach in Spain determined that the critical chilling models were at least 40% more efficient than the critical data model. Because in the critical chilling model, the chilling temperature was also calculated during the dormancy period. The results showed that the evolution of different phenological stages in the studied peach cultivars can be predicted with high efficiency by simple sequential statistical fitted models. In these models, chilling was evaluated by dynamic methods and forcing heat accumulation by growing degree sums. This model is applied to a wide range of climatic conditions of peach cultivation (Miranda et al., 2013).

In the study of the chilling and heat requirement of 63 nectarine genotypes and 118 peach genotypes over seven years, it was determined that the Luedeling model, compared to the Alonso model, was more successful in estimating the chilling and heat requirement in Argentina's climate conditions. In addition, chilling accumulation could be calculated using Chilling hours or positive Utah models (Maulion et al., 2014).

The study of peaches and nectarines in different climatic conditions showed that the chill accumulation in Murcia-Torre Pacheco (Spain) was 45 chill portions, in Coneo (Italy) and Bucharest (Romania), 97-98 chill portions. After earlier blooming in relatively cold areas and with a delay of 7-11 days in warmer areas, it can be explained that the bloom time is significantly affected by the delay of rest completion in those areas. The dynamic model performed better than others in predicting the bloom date (Drogoudi et al., 2023).

Another method for estimating chilling requirements in temperate trees, such as peach, can be using a portable E-nose. In the peach tree, the use of the E-nose method and the Partial Least-Squares Regression (PLSR) quantitative analysis model successfully estimated the chilling requirement. This method can also be used in other fruit trees (Yan et al., 2021).

(4) When endo dormancy ends and starts heat accumulation (eco dormancy)

There are more methods to estimate the point where to stop counting the chilling accumulation and when to start counting the heat amount:

- Take the shots in the laboratory and make observations in a controlled environment.

In the research of Milech et al. (2022), it was found that the Tabuenca test, which is based on the difference in the dry weight of the bud, has shown excellent and acceptable performance in estimating the end of dormancy in peaches. It has been explained that the d+ 7.2ºC and d+ 11ºC models are helpful and efficient in calculating chilling accumulation. According to the d+ 11ºC model, the number of hours required to complete dormancy for the BRS Bonão, Esmeralda, Granada, and Eragil cultivars was 180, 250, between 300 and 400 and more than 500 hours, respectively (Milech et al., 2022).

- Recurrent algorithms (Gaylen et al., 1977).

Where temperature and complete bloom data are available, but the end of rest data are not known, the constants can be accurately estimated with several steps through a recurrent algorithm. It starts with an estimation of the CU requirement, followed by an estimated date for the end of the rest. Calculate GDH accumulation until the observed date of full bloom for at least six years of data. Calculate the standard deviation for the GDH accumulations and repeat the steps.

(5) Heat accumulation models and patterns studied for different cultivars

Since 1730, when Reamur introduced the concept of the heating unit (McMaster & Wilhelm, 1997), many methods have been developed. GDD (growing degree days) and GDH (growing degree hours) are the most known.

GDD (growing degree days) collects the days when the average temperature exceeds a certain threshold specific to each species.

$GDD = Tavg – Tbase$, if $Tavg \geq Tbase$

$GDD = 0$, if $Tavg < Tbase$

McMaster & Wilhelm (1997) highlight the basic formula for GDD (Tmin./Tmax.).

GDH (growing degree hours) is defined by Richardson et al. (1974) as:

(1) $GDH = F*A/2 (1+\cos (\pi + \pi (TH-TB)/ (TU-TB)))$, where:

TH = hourly temperature,

TB = base temperature (4°C for trees),

TU = optimal temperature (25°C for trees)

TC = critical temperature (36°C in trees),

A = TU-TB,

F = a stress factor (due to biotic/abiotic factors). In most of the research until now, F was considered = 1

(for ex. Nutrition status, system planting - where the Vertical axis can lead to one-week harvesting airlines than Trident, etc.)

(2) GDH = F*A (1+cos (π/2 + π/2 (TH-TU)/(TC-TU)))

If TH < TU equation (1) is used, if TH>=TU, (2) (Gu, 2016).

How many GDH are needed to bloom (BBCH 67) or to be ready for harvesting (BBCH 87) for peach/ nectarine/ apricot for a specific cultivar?

In the literature are at least two methods: (1) take the shoots in the laboratory and make observations in a controlled environment (Valentini et al., 2004; Pantelidis et al., 2022); (2) make observations in the field and summaries with multiannual data (Richardson et al. (1975), Scalabrelli & Couvillon (1986), Roman et al. (1998), Citadin et al. (2001), Marra et al. (2002), Ruiz et al. (2006), Ruiz et al. (2007), Mounzer et al. (2008), Litschmann et al. (2008), Razavi (2011), Miranda et al. (2013), Guo et al. (2015), Kwon et al. (2020), Fadón et al. (2020), Rodriguez et al. (2021), Atagul et al. (2022), Drogoudi et al. (2023).

More details in research found were on the prediction of the harvest day correlated to GDH in the first 30 or 60 days after blooming:

- ❖ Peach and nectarine: correlation between GDH30 (Mimoun & DeJong, 1999) or GDH60 () with the harvest day.
- ❖ Determine the GDH30 and GDH60, and pruning can be applied to avoid smaller fruits if there is an earliness in the harvest time.

UC DAVIS – extension services for farmers:
(https://fruitsandnuts.ucanr.edu/Weather_Services/Harvest_Prediction__About_Growing_Degree_Hours/)

The researchers suggested that the blooming time is more affected by heat. As more heat accumulates, blooming in peach trees in Spain and Greece has also occurred earlier (Pantelidis et al., 2022). It has also been reported that heat accumulation after bloom is one of the critical factors in the harvest date and fruit development (Day et al., 2008).

Different cultivars of *Prunus persica* (L.) Batsch have different heat requirements for blooming and leafing. DellaNona and BR-1 cultivars had high heat requirements. However, a moderate heat requirement was observed in Planalto, Sunlite, and Eldorado cultivars. The heat requirement of Precoconho and Riograndense cultivars was also low compared to the other cultivars. During eco dormancy, the thermal requirements of vegetative and flower buds differed. Therefore, the results showed that prolonging chilling increased leafing compared to blooming (Citadin et al., 2001).

The study of different cultivars of apricot and peach showed that the cultivars' time to remove dormancy differed. This time in apricots was from late December to early February and in peaches from late December to mid-January. Furthermore, the results showed that the growing degree hour accumulations and growing degree day accumulations are reliable and effective methods for estimating the heat requirement in trees (Valentini et al., 2004).

The study of heat requirement in 136 peach cultivars for eight growing seasons showed that its range differed between the studied years from 1362 to 10,348 growing degree hours. This study observed a positive correlation between the bloom date and chilling requirement and a negative correlation between the chilling requirement and the heat requirement. In addition, it was found that the base temperature in each genotype must be determined accurately to determine the heat requirement accurately. The type of different genotypes of peach in the heat requirement provides the possibility of using them in breeding programs and introducing the cultivars capable of cultivation in regions with different climatic conditions (Atagul et al., 2022).

The study of 100-year data in Korea related to the chilling and heat requirement of peach cultivars showed that the chilling requirement of 15 investigated cultivars was between 263 and 2,123 chill hours, 377 and 1,134 chill units, 21.3 and 74.8 portions chilling. In the comparison between the models (Chill Hours, Utah, Dynamic, North Carolina, and Low Chilling models), it was also found that the dynamic model had the highest accuracy and the lowest changes between the years. In the next place was the Utah model. The range of heat requirement varied between 4,825 and 5,506 growing degree hours and was positively correlated with flowering time. During the 100 years studied, in the Utah and chill hours models, the start of chilling accumulation was delayed by 10 to 12 days (Kwon et al., 2020).

CONCLUSIONS

Fruit growing stages is an old research field with many steps made in the research network. Modeling the processes and transferring them in the deep learning machines were followed in more regions of the world.

Stone fruits were highly researched, and nowadays, accurate algorithms can be applied in different support decision systems with reasonable accuracy.

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GENETIC VARIABILITY STUDY OF ROMANIAN SWEET CHERRY GENOTYPES PRESENT IN THE USAMV OF BUCHAREST ORHARD COLLECTION

Mihaela IORDĂCHESCU[1], Cristina IONESCU[1], Liliana BĂDULESCU[2]

[1]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaela.iordachescu@qlab.usamv.ro

Abstract

Sweet cherry, Prunus avium L. belongs to the Rosaceae family, and nowadays is cultivated worldwide in temperate climates. Currently, plant breeders are using molecular markers, such as RAPD to reduce the time required to create new hybrids and valuable varieties. In the present study, seven RAPD markers were used to study the genetic variability and genetic relationships between 15 sweet cherry genotypes present in the orchard collection of USAMV Bucharest, 14 Romanian ('George', 'Ludovan', 'Bucium', 'Maria', Iașirom', Cociuvaș', 'Alexus', 'Andreiaș', 'Rubin', 'Severin', 'Paulică', 'Boambe de Cotnari', 'Cetățuia', and 'Putna') and the Swedish cultivar 'Rivan'. All RAPD markers proved to be polymorphic, allowing for amplification of 144 loci, with P63 amplifying the highest number of loci (27). The UPGMA dendrogram build on RAPD data grouped the genotypes studied into 2 clusters, one cluster containing mostly descendent of 'Van' and 'Boambe de Cotnari', and the second cluster grouping varieties with German genitors. The present study demonstrates the genetic variability among the Romanian genotypes present in the USAMV of Bucharest orchard collection.

***Key words**: genetic diversity, Prunus avium L., genotypes, Random Amplification of Polymorphic DNA.*

INTRODUCTION

The cultivation and use of cherries, *Prunus avium* L., dates back thousands of years, the earliest recorded use of cherries tracing back to the ancient Greeks and Romans (Dirlewanger et al., 2007). Evidence suggests that cherries were known to the Greeks as early as 300 BC, and they were a popular fruit among the Romans, who are credited with spreading cherries throughout Europe and parts of Britain. During the Middle Ages, cherry cultivation continued to be popular in Europe (Campoy et al., 2016; Livarda, 2008). European settlers brought cherry trees to North America in the 1600's, where they became established and started to be widely cultivated and later spread throughout the world (Iezzoni et al., 2017). Romania is among the top countries producing cherries worldwide, most of the commercial sweet cherry orchards being located in the south and north-east of the country (Bujdosó & Hrotkó, 2017)

Over time, selective breeding and agricultural practices have led to the development of the many varieties of cherries we have today. As cherry cultivation was so popular, many European *ex situ* collections include also wild relatives, since these can be a pool of extremely valuable genes, especially in the context of climate change (Antić et al., 2020; Quero-García et al., 2017). The ongoing climate changes force breeders to accelerate the creation of new cultivars that can withstand the new harsh conditions, and this is possible only by incorporating molecular techniques alongside traditional breeding methods (Vicente, 2022).

One of the above-mentioned molecular techniques is Random Amplification of Polymorphic DNA (RAPD) (Udriște & Bădulescu, 2019), developed in 1990 (Williams et al., 1990), that uses a single random decamer primer that will attach to both forward and reverse DNA strands, and will amplify the sequences between two annealing sites (Babu et al., 2021).

RAPD technique has multiple applications, from the study of genetic diversity among populations, varieties, species, genera, to checking the genetic stability of plants grown in vitro, study of genetic relationships among cultivars, and cultivar identification within collections (Antić et al., 2020; Ben Tamarzizt et al., 2015; Berindean et al., 2016; Bramhanapalli et al., 2017; Iancu & Chivu, 2021; Zarei et al., 2017).

The goal of the present study is to reveal the genetic variability and genetic relationships among fifteen sweet cherry accessions present in the USAMV of Bucharest orchard collection.

MATERIALS AND METHODS

Plant material

In the present study were used 15 sweet cherry accessions from the orchard collection of USAMV Bucharest, 14 Romanian (‘George’, ‘Ludovan’, ‘Bucium’, ‘Maria’, Iașirom’, Cociuvaș’, ‘Alexus’, ‘Andreiaș’, ‘Rubin’, ‘Severin’, ‘Paulică’, ‘Boambe de Cotnari’, ‘Cetățuia’, and ‘Putna’) and the Swedish variety ‘Rivan’ (Figure 1).

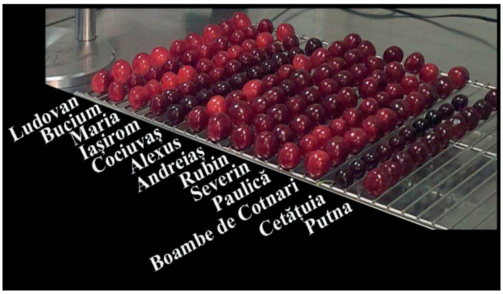

Figure 1. Fruits of 13 sweet cherry varieties

Genomic DNA extraction

Genomic DNA was extracted from young leaves using the Innu PREP Plant DNA I KIT IPC 16 Kit (Analytik Jena) according to the manufacturer instructions, based on the optimization reactions described before (Ionescu et al., 2022). Briefly, tissue was ground to powder with liquid nitrogen, then mixed with SLS lysis solution, and proteinase K, incubated for 1 hour at 60°C, centrifuged to remove plant tissue debris, supernatant treated with RNase A, and transferred to plate for automatic DNA extraction in the InnuPURE C16 System, using the Ext_Lysis_200_C16_04 program.

DNA quality and quantity were checked with Nanodrop 1000 (Biorad).

Measurement of DNA quantity and quality

DNA concentration and quality, based on the A260/A280 and A260/A230 absorbance ratios, were measured with the NanoDrop 1000 spectrophotometer (Biorad).

RAPD reaction

Polymerase chain reaction (PCR) was done with the Platinum™ II Hot-Start PCR Master Mix (2X) (Invitrogen) according to the manufacturer’s instructions. PCR reactions were set up in a 10 µl total volume, a final concentration of 2 ng/µl RAPD primer, and 1 ng/µl genomic DNA. For all primers used the annealing step was done at 32ºC. The seven RAPD primers used were P59, P60, P61, P63, P64, P65, and P66, their sequence being presented in Table 1.

Table 1. RAPD primers nucleotide sequence

Primer	DNA sequence
P59	5’-GTTGGTGGCT-3’
P60	5’-GGGAACGTGT-3’
P61	5’-CCGTGACTCA-3’
P63	5’-TGCCGAGCTG-3’
P64	5’-AGGTGACCGT-3’
P65	5’-GTTGGTGGCT-3’
P66	5’-GGGAACGTGT-3’

DNA fragments amplified by the RAPD reactions were separated on 1.5% agarose gel and visualized with the Pharox FX system (BioRad).

Data analysis

The lengths of the amplicons were measured with the Quantity One software (Version 4.6.9., BioRad), based on the 1 Kb Plus DNA Ladder (Invitrogen). Data were converted into a binary matrix and scored as present (1) or absent (0) in a *.csv file, and later analysed with the BIO-R software (Biodiversity Analysis with R for Windows), version 3.0.

RESULTS AND DISCUSSIONS

All decamer primers were polymorphic, and the DNA fragments amplified by PCR had lengths between 170 and 2950 bp, with total number of 144 loci (Table 2). The highest number of loci were observed for the primer P63 (27), and the lowest number of loci was observed for the primer P64 (12).

Table 2. The number of loci and amplicons' lengths corresponding to each decamer primer used

Primer	Number of f loci	Amplicon sizes (bp)
P59	20	1500, 1450, 1200, 1050, 1000, 870, 810, 770, 730, 700, 670, 630, 610, 580, 560, 530, 480, 440, 410, 390
P60	24	2950, 2800, 2400, 2160, 1920, 1800, 1750, 1600, 1500, 1300, 1200, 970, 880, 820, 750, 650, 610, 560, 470, 430, 400, 320, 280, 250
P61	25	2450, 2170, 2000, 1700, 1620, 1530, 1400, 1350, 1250, 1060, 940, 900, 800, 740, 670, 620, 550, 500, 450, 400, 350, 300, 250, 200, 170
P63	27	2670, 2280, 2100, 1990, 1820, 1750, 1650, 1500, 1400, 1300, 1200, 1170, 1050, 900, 870, 810, 760, 740, 700, 650, 610, 550, 530, 480, 420, 400, 360
P64	12	2300, 1500, 1150, 1050, 870, 680, 600, 570, 470, 440, 300, 200
P65	17	2550, 2100, 1800, 1420, 1200, 1100, 950, 850, 700, 600, 550, 500, 430, 400, 350, 320, 300
P66	19	270, 2050, 1850, 1600, 1500, 1260, 1160, 1150, 1060, 900, 850, 730, 630, 590, 530, 440, 400, 220, 190

The calculated Roger's genetic distances are represented in Table 3. The shortest genetic distance, 0.48, is observed between the 'Rivan' and 'Rubin' varieties, whereas the most distantly related are the varieties 'Alexus' and 'Ludovan', followed by 'Alexus' and 'Cetățuia', as it can be also observed from the dendrogram presented in Figure 2.

Table 3. Roger distances calculated with the Bio-R software

NAME	George	Ludovan	Bucium	Maria	Iașirom	Cociuvaș	Alexus	Andreiaș	Rubin	Severin	Paulică	Boambe de Cotnari	Cetățuia	Putna	Rivan
George	0.00	0.57	0.52	0.60	0.51	0.53	0.64	0.59	0.60	0.54	0.53	0.60	0.60	0.61	0.60
Ludovan	0.57	0.00	0.55	0.51	0.61	0.53	0.69	0.63	0.65	0.62	0.57	0.61	0.54	0.61	0.61
Bucium	0.52	0.55	0.00	0.58	0.53	0.51	0.63	0.61	0.60	0.56	0.56	0.60	0.57	0.61	0.57
Maria	0.60	0.51	0.58	0.00	0.62	0.55	0.65	0.65	0.62	0.62	0.55	0.63	0.61	0.62	0.58
Iașirom	0.51	0.61	0.53	0.62	0.00	0.54	0.63	0.54	0.56	0.60	0.59	0.58	0.60	0.58	0.58
Cociuvaș	0.53	0.53	0.51	0.55	0.54	0.00	0.60	0.59	0.61	0.55	0.51	0.61	0.61	0.65	0.59
Alexus	0.64	0.69	0.63	0.65	0.63	0.60	0.00	0.57	0.59	0.60	0.61	0.60	0.67	0.61	0.55
Andreiaș	0.59	0.63	0.61	0.65	0.54	0.59	0.57	0.00	0.58	0.59	0.60	0.58	0.57	0.61	0.57
Rubin	0.60	0.65	0.60	0.62	0.56	0.61	0.59	0.58	0.00	0.55	0.58	0.53	0.60	0.60	0.48
Severin	0.54	0.62	0.56	0.62	0.60	0.55	0.60	0.59	0.55	0.00	0.57	0.58	0.62	0.60	0.54
Paulică	0.53	0.57	0.56	0.55	0.59	0.51	0.61	0.60	0.58	0.57	0.00	0.57	0.63	0.62	0.53
Boambe de Cotnari	0.60	0.61	0.60	0.63	0.58	0.61	0.60	0.58	0.53	0.58	0.57	0.00	0.58	0.60	0.51
Cetățuia	0.60	0.54	0.57	0.61	0.60	0.61	0.67	0.57	0.60	0.62	0.63	0.58	0.00	0.49	0.58
Putna	0.61	0.61	0.61	0.62	0.58	0.65	0.61	0.61	0.60	0.60	0.62	0.60	0.49	0.00	0.55
Rivan	0.60	0.61	0.57	0.58	0.58	0.59	0.55	0.57	0.48	0.54	0.53	0.51	0.58	0.55	0.00

The colour gradient ranges from Blue to Red, short to long distance

The UPGMA dendrogram built with the Bio-R software grouped the genotypes into two main clusters, the first one with 7 varieties, and the second one with 8 varieties (Figure 2).
In the first cluster, 'Bucium' and 'Cociuvaș' are grouped together having as common genitor 'Boambe de Cotnari'. 'Ludovan' and 'Maria' have the common genitor the variety 'Van'. In addition, four out of seven varieties in this cluster have as genitor the variety 'Van'. The varieties present in the second cluster have a larger pool of genitors, however many of these

are of German origin, and again the 'Cetățuia' and 'Putna' varieties, having as common genitor 'Van', are grouped together.

Table 4. Varieties' origin

Nr. crt.	Variety	Origin
1.	George	'Cireșe de octombrie' x 'Fromm'*
2.	Ludovan	'Van' x 'Boambe de Cotnari'*
3.	Bucium	'Van' x 'Boambe de Cotnari'*
4.	Maria	'Van' x 'Stella'*
5.	Iașirom	'Van' x 'Boambe de Cotnari'*
6.	Cociuvaș	'Boambe de Cotnari' x 'Bigarreau Moreau'*
7.	Alexus	Polenizare liberă 'Lijana'*
8.	Andreiaș	HC.27/4 x Boambe de 'Cotnari'*
9.	Rubin	'Hedelfinger' x 'Germersdorf'*
10.	Severin	'Thurn und Taxis' x 'Germersdorf'*
11.	Paulică	'Bigarreau Drogan' x 'Fromm'*
12.	Boambe de Cotnari	Local selection**
13.	Cetățuia	'Van' x 'Boambe de Cotnari'*
14.	Putna	'Van' x 'Muncheberger Fruhe'*
15.	Rivan	'Early Rivers' x 'Van'***

*(Ștefan et al., 2018)
**(Blaja et al., 1965)
***(Budan & Grădinariu, 2000)

It is interesting to note that in the first cluster, three varieties, 'Iașirom', 'Bucium', and 'Ludovan' have the same genitors: 'Van' x 'Boambe de Cotnari', however they are separated in the three subclusters.
In addition, the 'Cetățuia' variety located in the second cluster also has as genitors 'Van' x 'Boambe de Cotnari'.
These apparent differences among close relatives could be because RAPD markers are dominant, so they cannot differentiate between homozygous and heterozygous loci, and furthermore, amplicons of similar lengths do not have necessarily the same DNA sequence (Amiteye, 2021), so some varieties may appear closer related than they really are.
Nevertheless, for the purpose of this study, the seven RAPD markers did highlight the genetic variability among the varieties studied and generated genetic fingerprints variety-specific.

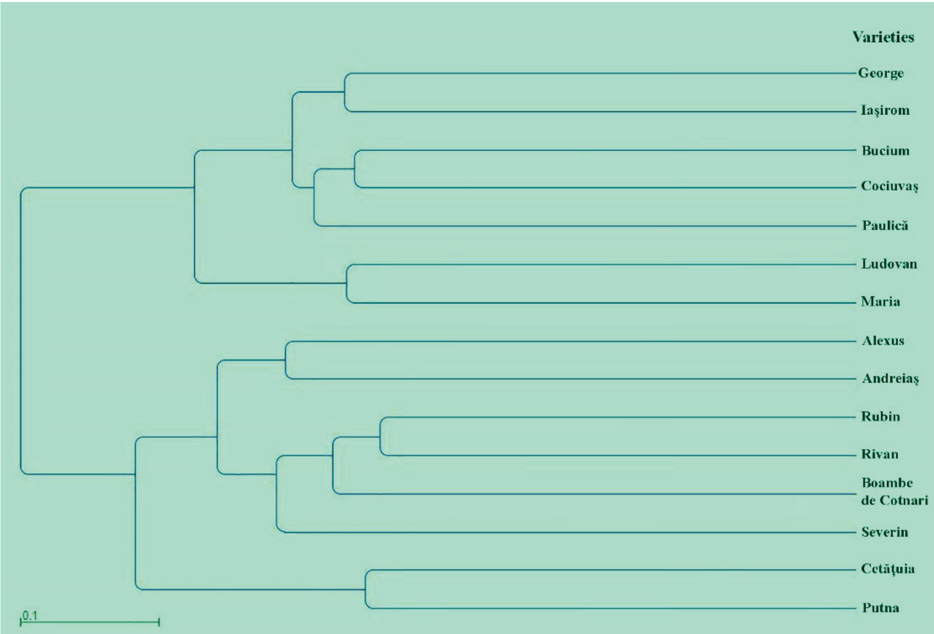

Figure 2. Dendrogram based on the RAPD data generated with the Bio-R software

CONCLUSIONS

RAPD analysis of the fifteen accessions from the USAMV of Bucharest orchard revealed that:

- ✓ All random decamer primers identified polymorphic RAPD loci.
- ✓ P63 marker identified the highest number of loci, making it the most suitable marker to discriminate among the fifteen accessions.
- ✓ Analysis of the data generated from the RAPD reactions using the seven decamers was used to construct a UPGMA

dendrogram, and to demonstrate the genetic variability among the cultivars studied.

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RESEARCH ON RESISTANCE OF SOUR CHERRY CULTIVARS IN DROUGHT CONDITIONS IN THE NORTHEASTERN AREA OF ROMANIA

Elena IUREA, Sorina SÎRBU, Iulia MINEAȚĂ, Iuliana Elena GOLACHE, Mădălina GHERGHEL, Margareta CORNEANU

Research Station for Fruit Growing Iasi, 3 Ion Vodă cel Viteaz Street, Iasi, Romania

Corresponding author email: sorinas66@yahoo.com

Abstract

This paper presents aspects recorded in the area of influence of the Research Station for Fruit Growing Iasi during 2020-2022 on five Hungarian sour cherry cultivars, able to capitalize on the Romanian agroclimatic conditions. Analysing from a water stress point of view, the April-July time interval when the intensive growth of shoots took place, during the studied years there was a deficit of -54.2 mm in 2020, -11.2 mm in 2021 and -113.8 mm in 2022 compared to the multiannual amount for this period. Analysing the trunk section area in terms of the average of the three years of study, the values were between 10.5 cm^2 ('Erdi Ipari') and 24.9 cm^2 ('Erdi Bibor'). The highest values for the crown volume were recorded in the cultivars 'Erdi Kordi' (4.53 m^3/tree) and 'Dukat' (4.15 m^3/tree) and the lowest crown volume was recorded in the cultivars 'Erdi Bibor' (3.78 m^3/tree), 'Erdi Kedves' (3.27 m^3/tree) and 'Erdi Ipari' (2.20 m^3/tree). The density of the tree crown recorded values between 4.45 cm^2/m^3 ('Erdi Ipari') and 9.28 cm^2/m^3 ('Erdi Bibor').

Key words: *crown volume, deficiency, measurements, precipitation, shoots.*

INTRODUCTION

The role of water as a vegetation factor is decisive in the life of plants, representing the main constituent of plant organisms. Water is the vehicle of nutrients and the main thermal regulator (Budan & Grădinariu, 2000; Asănică & Hoza, 2013).

During the growing season, the water consumption of fruit-growing trees is variable. Thus, the critical phases of the vegetation period when the water consumption is maximum are the growth of shoots, the flowering time and the growth of fruits (Toma & Robu, 2000; Ghena & Branişte, 2003; Toma & Jităreanu, 2007).

In the area of influence of the Research Station for Fruit Growing Iasi, the low rainfall throughout the year causes great damage in the sour cherry orchards. The lack of water determines a reduced vigour of the trees, disorder of the regularity of the fruits production and size of the harvests, the aging and shortening of the growth and development stages of the plants, gum discharges (gummosis) and physiological imbalance with pathogenic aspect (Milică et al.,1982; Vasilev et al., 1982; Sestraş, 2004).

The years 2020-2022 can be characterized as years with special climatic peculiarities that have negatively influenced the annual twigs and fruit production in this area.

In this paper we present some aspects recorded in the area of influence of the Research Station for Fruit Growing Iasi during 2020-2022 on some sour cherry cultivars, able to capitalize on the existing agroclimatic conditions.

MATERIALS AND METHODS

The studies were carried out between 2020 and 2022, having as research material five sour cherry cultivars coming from Hungary ('Erdi Ipari', 'Erdi Kedves', 'Erdi Bibor', 'Erdi Kordi', and 'Dukat'), which are in the sixth year after planting. The trees are grafted on *Prunus mahaleb* L., guided as freely flattened palmette. The plantation is placed randomly, in three repetitions of two trees per repetition, at 3.5 x 4 m resulting 714 trees per ha. On the row with trees, the soil was prepared with the rotary orchard tiller and between the rows the soil was grassed.

The land on which the plantation was established was in the Jijia-Bahlui depression, where the multiannual average temperature was 10.2°C.

The meteorological factors (during the three years of study) were analysed along with the behaviour of the cultivars towards the drought that took place during the studied period and biometric measurements and determinations of the tree and of the shoots were performed (the trunk section area (TSA), the height of the tree (H), the volume of the crown (CV), the length of the annual twigs (LCA) and their amount on the tree, the density of the crown (CD).

The height of the tree (H) and the length of the annual twigs (LCA) were measured with the measuring roulette, the trunk section area (TSA) was measured by using the calliper on the thickness of the trunk in two perpendicular directions 30 cm high from the grafting point (and the data taken at TSA were transformed into cm^2). In order to calculate the average volume (m^3) of the crown of the trees, the Sarger method was used with the formula: $\mathbf{V = (D + d/2)^2 \times H \times 0.416}$, in which: CV - the volume of the crown, D – the diameter of the crown in the direction of the row of trees, d - the average diameter of the thickness of the fruiting hedge, H - the height of the trees, 0.416 - the correction coefficient (Lamureanu et al., 2013), the density of the crown was calculated according to the formula: **TSA (cm^2)/ CV (m^3)** (Parnia & Mladin, 1995).

The experimental data were interpreted statistically by the variance analysis and the method of multiple comparisons (Duncan test, with P 5%). The determination of the correlation coefficient (r) was performed using Bravais's formula (Timariu et al., 1978).

RESULTS AND DISCUSSIONS

In sour cherry, the requirements for water increase starting with April and they rich the highest point in the months of May-July, when the intensive growth of the shoots takes place (Roversi & Ughini, 1993), then in August when the growth of the shoots ceases (the synthesis of carbohydrate substances, proteins, etc. takes place), followed by their thickening and at the same time the differentiation of the buds begins (Teaci et al., 1982; Grădinariu & Istrate, 2003).

The average annual temperatures recorded had the following values: 12.1°C in 2020, 10.1°C in 2021, and 11.3°C in the first seven months of 2022 (the multiannual average being 10.2°C). Analysing the same period for the rainfall parameter, the years 2020 (448.4 mm) and 2022 (142.4 mm the first seven months of the year) recorded quantities below the multiannual limit (562.6 mm), achieving a deficit of 114.2 mm in 2020 and 266.5 mm during January-July 2022. More, in 2021, the quantity of rainfall was close to the multiannual average (Table 1).

From the analysis of the trunk section area (TSA) in the sixth year (2022) after planting it was found that the largest TSA was recorded in the 'Erdi Bibor' cultivar (35.1 cm^2), and the smallest in 'Erdi Ipari' (9.8 cm^2).

Analysing this parameter in terms of the average of the three years of study, it can be noticed that they do not have statistically insured values, thus, negative differences in comparison with the average of the variants (19.4 cm^2) were recorded by the cultivars 'Erdi Ipari' (10.5 cm^2), 'Erdi Kedves' (19.1 cm^2) and 'Dukat' (19.3 cm^2), and the cultivars 'Erdi Bibor' (24.9 cm^2), and 'Erdi Kordi' (23.0 cm^2) recorded positive differences in comparison with the average of the variants (19.4 cm^2) (Table 2).

Correlating these parameters for the trees in their 6^{th} year since planting resulted in a positive correlation coefficient (R^2= 0.7049), statistically non-significant (Figure 1). Low vigour of the tree is an important parameter for establish high density of the orchards, the new trends in the fruit tree growing being to increase constant the productivity (Stănică, 2019). From our results, the cultivars 'Erdi Ipari', 'Erdi Kedves', and 'Dukat' are suitable for higher densities per hectare.

Analysing the crown volume (CV), the highest values were recorded in the cultivars 'Erdi Kordi' (4.53 m^3/ tree, 3234.4 m^3/ha) and 'Dukat' (4.15 m^3/tree, 2963.1 m^3/ha), and the lowest volume of the crown was recorded by the cultivars 'Erdi Ipari' (2.20 m^3/tree, 1570.8 m^3/ha), 'Erdi Kedves' (3.27 m^3/tree, 2334.7 m^3 /ha), and 'Erdi Bibor' (3.78 m^3/tree, 2698.9 m^3/ha) (Table 2). According to Kiprijanovski et al. (2018), crown sizes depend on lots of factors, like rootstock vigorousness, planting distance, or the correction by pruning.

The density of the tree crown (CD) had values between 4.45 cm^2/m^3 ('Erdi Ipari') and 9.28 cm^2/m^3 ('Erdi Bibor') (Table 2).

Table 1. Climatic characterization of the years 2020-2021-2022

Year/Month			January	February	March	April	May	June	July	August	September	October	November	December	Annual sum (mm)	Annual average (°C)
Monthly sum of the precipitations (mm)	**Multiannual average**		**35.5**	**32.1**	**71.2**	**51.4**	**71.1**	**82.9**	**64.7**	**50.8**	**36.5**	**2.4**	**33.4**	**30.6**	**562.6**	x
	2020	Monthly sum	3.6	40.4	17.4	0.8	67.8	82.6	32.8	13.4	30.2	85.2	17.8	56.4	448.4	x
		Deviation	-31.9	+8.3	-53.8	-50.6	-3.3	-0.3	-31.9	-37.4	-6.3	+82.8	-15.6	+26.2	**-114.2**	x
	2021	Monthly sum	24.0	23.6	52.2	46.6	70.4	99.6	55.4	127.2	11.6	4.4	9.6	39.0	563.6	x
		Deviation	-11.5	-8.5	-19.0	-4.8	-0.7	+16.7	-9.3	+76.4	-24.9	+2.0	-23.8	-8.4	**+1.0**	x
	2022*	Monthly sum	6.6	10.4	6.0	58.0	17.4	16.2	27.8	-	-	-	-	-	142.4	x
		Deviation	-28.9	-21.7	-65.2	+6.6	-53.7	-66.7	-36.9	-	-	-	-	-	**-266.5**	x
Average air temperature (°C)	**Multiannual average**		**-1.9**	**-1.2**	**4.7**	**11.4**	**17.0**	**20.5**	**22.4**	**21.9**	**16.3**	**5.4**	**5.4**	**0.1**	**x**	**10.2**
	2020	Monthly average	0.9	4.2	7.6	11.5	14.0	20.9	22.6	23.6	19.6	13.9	4.5	2.0	x	+12.1
		Deviation	-1.0	-3.0	+2.9	+0.1	-3.0	+0.4	+0.2	+1.2	+3.3	+8.5	-0.9	+1.9	x	**+1.9**
	2021	Monthly average	0.1	-0.6	3.4	8.2	15.1	19.6	23.2	20.9	14.9	9.4	6.5	0.6	x	+10.1
		Deviation	-1.8	-0.6	-0.3	-3.2	-1.9	-0.9	+0.8	-1.0	-1.4	+4.0	+1.1	+0.5	x	-0.1
	2022*	Monthly average	0.4	3.7	3.2	10.0	16.6	21.9	23.2	-	-	-	-	-	**x**	+11.3
		Deviation	-1.5	+2.5	-1.5	-1.4	-0.4	+1.4	+0,8	-	-	-	-	-	**x**	**+1.1**

*In 2022, the data for the first seven months of the year (January-July) was displayed

Table 2. Tree growth vigour and crown volume for some sour cherry cultivars in the sixth year after planting (RSFG Iasi; average of 2020-2022)

Cultivar	Trunk section area (cm^2)		Crown volume in the 6th year (m^3)		Crown density cm^2/m^3
	Year VI*	Average of the years IV-VI	per tree	per ha	
Erdi Bibor	35.1^{a}	24.9	3.78^{b}	2698.9	9.28^{a}
Erdi Kordi	30.8^{ab}	23.0	4.53^{a}	3234.4	6.80^{b}
Dukat	28.3^{b}	19.3	4.15^{ab}	2963.1	6.82^{b}
Erdi Kedves	27.9^{b}	19.1	3.27^{b}	2334.7	8.53^{ab}
Erdi Ipari	9.8^{c}	10.5	2.20^{bc}	1570.8	4.45^{bc}
Average (x)	***26.4***	***19.4***	***3.59***	***2560.3***	***7.18***
DL 5% DL 1% DL 0.1%	DL 5% = 1.74	6.4 9.2 13.9	DL 5% = 0.22		DL 5% = 0.05

*- different letters correspond with the significant statistical difference for $P \leq 5\%$, Duncan test.

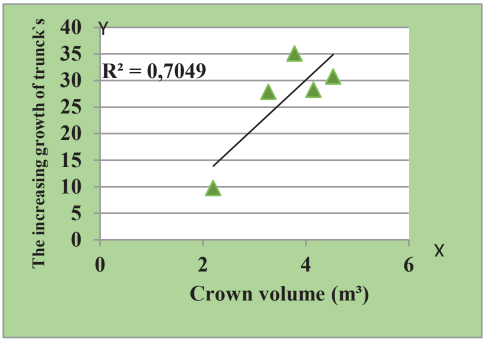

Figure 1. Correlation between trunk section area (cm^2) and crown volume (m^3)

In August, when the growth of the shoots ceased (the apical bud is outlined at the top of the shoots), the length of the annual twigs in the studied sour cherry cultivars were counted and measured.

Analysing from a water stress point of view, the April-July time interval when the intensive growth of shoots takes place, in the studied years there was a deficit of -54.2 mm in 2020, of -11.2 mm in 2021, and of -113.8 mm in 2022 in comparison to 270.1 mm representing the multiannual amount for this period (Table 1).

It is known that the lack of water reduces the number and growth in length of annual shoots, the photosynthesis, the transport of substances in the plant and decreases the turgidity of cells (Toma & Jităreanu, 2007). According with that we recorded the number of annual twigs per tree in conditions of the average of the three years of study (2020-2022) and we observe that the cultivars 'Erdi Bibor' with 58 twigs/tree, 'Erdi Kordi' with 49 twigs/tree and 'Erdi Kedves' with 45 twigs/tree got highlighted with the highest amount of twigs per tree, recording positive differences in comparison with the average of the cultivars (43 pcs.). But, the cultivars 'Dukat' with 26 twigs/tree and 'Erdi Ipari' with 36 twigs/tree got highlighted as the cultivars with the smallest amount of twigs per tree, recording negative differences in comparison with the average of the cultivars (Table 3). Mika et al., 2011, showed that the presence of the annual and two-year-old shoots are favourable to the abundant setting of flower buds and to flowering so after pruning should remain only these younger branches for a good production.

Correlating the mean of the IV-VI years of the trunk section area (cm^2) with the mean of the IV-VI years of the amount of annual twigs on tree (pcs), it was noticed that the two parameters are positively correlated (R^2 = 0.3797), however statistically non-significant (Figure 2).

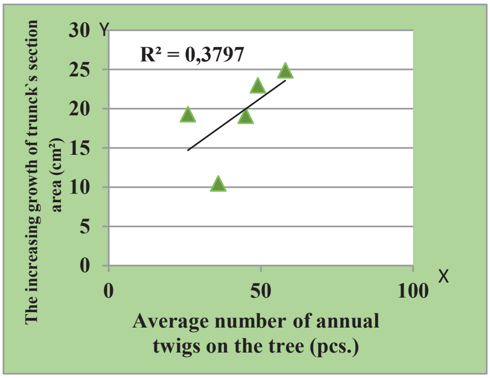

Figure 2. Correlation between trunk section area (cm^2) and the amount of annual twigs/tree (pcs.)

Analysing the average length of the annual twigs in the studied three years, this parameter oscillated between 45.7 cm ('Erdi Ipari') and 69.6 cm ('Dukat'), statistically, the 'Dukat' cultivar recording positive differences in comparison with the average of the cultivars (56.2 cm) (Table 3). Correlating the mean of the IV-VI years for the trunk section area (cm^2) with the mean of the IV-VI years for the annual twigs length (cm), it was noticed there was a positive correlation between the two parameters (R2 = 0.2076), statistically non-significant (Figure 3). Considering that during May-July when the growth and development of the tree shoots takes place, the rainfall was reduced (in comparison with the multiannual average for this period (218.7 mm), the deficit was -35.5 mm in 2020, -6.7 mm in 2021, and -157.3 mm in 2022), the entire physiological activity of the tree was reduced, recording reduced values for the studied vegetative pomological parameters.

Table 3. Data on the number of annual twigs per tree and their average length for some sour cherry cultivars in the sixth year after planting (RSFG Iasi; average of 2020-2022)

Cultivar	Average number of annual twigs on the tree (pcs.)				The average length of annual twigs (cm)			
	Year IV	Year V	Year VI	Average of the years IV-VI*	Year IV	Year V	Year VI	Average of the years IV-VI
Erdi Bibor	38[++]	52	84	58[a]	45.3	84.2	43.4	55.6[ab]
Erdi Kordi	17	52	78	49[ab]	25.0	91.3	49.2	55.4[b]
Erdi Kedves	18	37	81	45[b]	21.2	92.7	53.5	54.5[b]
Erdi Ipari	14	36	59	36[bc]	20.7	75.0[000]	44.8	45.7[b]
Dukat	15	28	36	26[c]	37.7	120.0[++]	62.7	69.6[a]
Average (x)	***20***	***41***	***67***	***43***	***30.0***	***92.6***	***50.7***	***56.2***
DL 5% DL 1% DL 0.1%	11.7 17.0 25.5	16.1 23.4 35.2	49.7 72.4 108.6	DL 5% = 5.44	22.6 32.9 49.4	17.6 25.7 38.5	18.1 26.4 39.6	DL 5% = 5.96

*- different letters correspond with the significant statistical difference for P ≤ 5%, Duncan test.

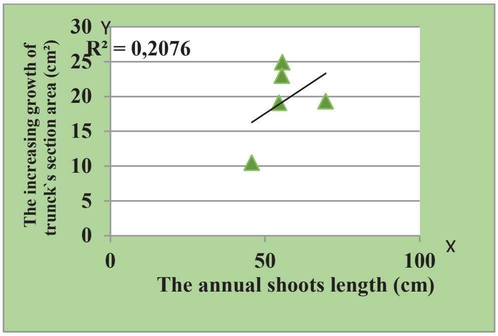

Figure 3. Correlation between trunk section area (cm^2) and annual shoots length (cm)

According to rainfall amount in the studied period (Table 1) we observed that 'Erdi Bibor' recorded an higher number of annual twigs (Table 3) on the tree in conditions of 2021 and 2020, while 'Dukat' recorded an higher length of annual twigs in conditions of 2021.

CONCLUSIONS

The research carried out in a period characterised by great climatic fluctuations, prove that the studied sour cherry cultivars have a good resistance to the agroclimatic conditions recorded during the studied period in the North-East area of Romania.
Analysing the values recorded for all the determined parameters, it was found that the five sour cherry cultivars in the sixth year after planting, have different biological traits of growth.

Regarding the volume of the crown of the trees, it was estimated that the smallest crowns were presented in the cultivars 'Erdi Ipari', 'Erdi Kedves', and 'Erdi Bibor' which had a tight growth of the crown.
Under the conditions of dry years, the cultivars highlighted as the most resistant to drought (with a large number of annual twigs on the tree and high values for their length) were 'Erdi Bibor', 'Erdi Kordi', and 'Erdi Kedves'.

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EARLY VEGETATIVE AND GENERATIVE DEVELOPMENT CHARACTERISTICS OF WILTON`S RED JONAPRINCE® SELECT ECO AND GOLDEN REINDERS® CULTIVARS ON B9 AND M9 DWARF ROOTSTOCKS IN IRRIGATED MOLIC EUTRICAMBOSOIL IN NORTHERN TRANSYLVANIA, ROMANIA

Zsolt JAKAB-ILYEFALVI[1], Larisa-Daniela VLAŞIN[1], Anca Maria CHIOREAN[1, 2], Smaranda ROŞU-MAREŞ[1, 2]

[1]Fruit Research & Development Station Bistrita, Drumul Dumitrei Nou, no. 3, Bistrita, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăştur Street, Cluj-Napoca, Romania

Corresponding author email: zsolt.jakab@yahoo.com

Abstract

The research revealed some important initial vegetative and generative characteristics and tendencies in the early years of development of Wilton`s Red Jonaprince Select Eco ® /B9 (WRJSE) and Golden Reinders/M9 (GR) cultivar-rootstock combinations in a field trial located at FRDS Bistrita, Northern Transylvania in a high density drip irrigated orchard. The strongest vegetative growth was observed in Wilton`s Star Red Jonaprince Select ECO®/B9 in comparison with Golden Reinders/M9 but on the other hand being young trees at early stages of development the before mentioned cultivars gave similar results regarding trunk cross sectional area, tree height. The highest generative bud ratio was found at GR, highest crop load was found on GR while the highest number of fruit per tree was found in GR and the highest fruit diameter was found on WRJSE in comparison with GD. Being in the first years of development the obtained yield/tree had close values (1.99 kg/tree at WRJSE and 1.95 kg/tree at GD), thus yielding a total production of 2.44-2.49 to/ha at WRJSE and GD.

Key words: *apple, rootstocks, irrigation, yield, vegetative parameters, generative parameters.*

INTRODUCTION

Low vigour rootstocks present a series of advantages related to productivity and precocity but require very good soils and adequate fertilization-irrigation systems.

The rootstock M9-T337 (M9) (dwarf) is the standard for the apple industry and breeding programs and Bud-9 (B9) (dwarf) is one of the most dwarfing commercially available rootstocks.

B9 rootstock (sin Budagovsky 9, Bud.9) is a dwarfing rootstock resulting from a cross of M.8 x Red Standard (Krasnij Standard) from Russia. The leaves are a distinctive red. Regarding the height, B.9 is slightly more dwarfing than M9, about 25-35%, and has slightly higher yield efficiency than M9. B9 appears to be resistant to collar rot and is very cold-hardy. It has performed very well across a wide range of conditions (Gonda, 2003; Sharma et al., 2009). Trees needed to be supported.

The American strain was slightly less dwarfing and produced more burr knots than the European strain and yield efficiency was similar (Robinson et al., 2007; Russo et al., 2007). M9 (sin. Malling 9, Paradis Jaune de Metz) is the traditional and best-known dwarfing rootstock. It should be planted on a well-drained site. Trees on this rootstock always require leader support (Dayatilake and van Hooijdonk, 2015). The rootstock is susceptible to fire blight and can develop burr knots (Russo et al., 2007). Numerous clones of M9 are now being sold by nurseries such as Pajam 1 (Lancep) and Pajam 2 (Cepiland) which are French selections relatively new. They are 35 to 40 % more vigorous than M9 NAKB 337.

High-density systems rely mainly on the rootstock M9, a highly productive dwarfing rootstock, which is particularly susceptible to rootstock blight. In heavy fire blight years under natural conditions, tree losses greater than 50% are common for orchards planted on M9 rootstock (Ferree et al., 2002; Robinson et al., 2007). According to the Regulation of the Commission of the European Communities No 85/2004 of 15 January 2004, which lays down the marketing standard for apples (European Union, 2011), Jonagold/Jonagored is classified with its mutants, including Crowngold, Decosta, Jonabel, Jonagold 2000, Jonagored Supra, King Jonagold, and Red Jonaprince. The production of Red Jonaprince in the European Union is rapidly increasing, and even the United States Department of Agriculture, while presenting apple production in European Union, indicates this specific mutant with Jonagold/Jonagored as a group of Jonagold/Jonagored/Red Jonaprince (Podbielska et al., 2017).

The cultivar Golden Delicious especially his mutant Golden Delicious Reinders which is free from russeting is one of the more promising cultivars, whose production in commercial orchards is increasing each year (Kruczyńska, 2008; Czynczyk and Bielicki, 2012).

In the experiment of Czynczyk and Bielicki (2006), Golden Delicious Reinders come into biennial bearing quite early. It is known from the foreign literature (Crassweller et al., 2001; Bonany et al. 2002), that all growers of Golden Delicious Reinders, in order to obtain good size and regularly yielding trees, have to thin fruitlets very heavily using bioregulators, which is often corrected by hand, so this involves hand work. To obtain good results, a lot of attention has to be paid to pruning and thinning of fruitlets (Czynczyk and Bielicki, 2012).

In this context, the paper presents an analysis of the vegetative and generative parameters in the North East region of Romania of Wilton`S Red Jonaprince® Select Eco grafted on B9 and Golden Delicious Reinders® grafted on M9 rootstocks, in order to put into evidence the comparison of the shoot growth, tree height, and trunk cross-sectional area. Regarding generative parameters, the bud ration, crop load, number of fruits, and their diameter were studied.

MATERIALS AND METHODS

The experiment was conducted 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 of around 10°C and a multiannual average of 758.80 mm of rainfall, according to the data recorded by the meteorological station at FRDS Bistrita, in the last 30 years. The climate is temperate-continental, with relatively hot summers, and less dry cold winters. The orchard was established in 2020, with Wilton`s Red Jonaprince Select Eco®/B9 (WRJSE) and Golden Reinders®/M9 (GR) apple trees in a high-density slender spindle training system with drip irrigation. The experiment had a bifactorial design using for Factor A the scion cultivars WRJSE and GR respectively for Factor B the rootstocks M9 and B9 as graduations. In our study during 2021-2022, we focused on three vegetative growing parameters (trunk cross-sectional area, tree height, shoot length) and six generative parameters (yield per tree, fruit number per tree, crop load, fruit diameter, fruit surface colour, brix degree content of soluble solids). Fruit surface colour was determined with application Image Analysis Toolset - IAT by Google, and it's method is colorimeter RGB. 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

Early results showed that the planted young trees with preformed crown produce vegetative and generative organs well balanced in the first years of growing with a good premise for yield (Figure 1).

Figure 1. Aspects from the high-density orchard Wilton`S Red Jonaprince® Select Eco/B9

From the first years of development, the trees produced fruits and the soil substrate until now seemed acceptable for M9 and B9 rootstocks in molic eutricambosoil (Figure 2), the root system explored well the upper surface of the soil.

Figure 2. Aspects from the high-density orchard with Golden Delicious Reinders®/M9

Regarding the meteorological conditions of the Bistrita area, the temperatures recorded in the last three years fluctuated, influenced by the complex factors of global climate changes. Annual average temperatures were between 10.5°C (2019)-11.2°C (2022), with absolute maximum temperatures between 34.6°C (2019) ranging 35.6°C (2022). Regarding rainfall, the total was 538.9 mm in 2019, 678.8 mm in 2020, 784.5 mm in 2021 and 759.3 mm in 2022 (Table 1). The average value of annual rainfall in the Bistrita region is 758.8 mm, but the years 1999 and 2000 were very dry compared with 2021-2022. A general observation during the last years is that the rainfall distribution is uneven during the year, which can cause an imbalance of growth and fructification processes for apple trees in non-irrigated plots. We concluded that the lack of necessary water amount was especially in the summer months June-August where drip irrigation is compulsory. In the drip irrigated research field, the trees became the required necessary water calculated by FAOSTAT ETP Calculator aimed by Pennmann-Monteth ETr formula for the development and bearing processes.

Table 1. Climate indicators 2019-2022

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

The strongest **vegetative growth** (Figure 3) was observed in Wilton`s Star Red Jonaprince Select ECO®/B9 (36.27 cm shoot lenght) in comparison with Golden Reinders®/M9 (26.36 cm shoot length), but on the other hand being very young trees the before mentioned cultivars gave quite similar results regarding trunk cross sectional area (Figure 4), 5.79 cm^2 in WRJSE and 5.61 cm^2 in GR in our preliminary research.

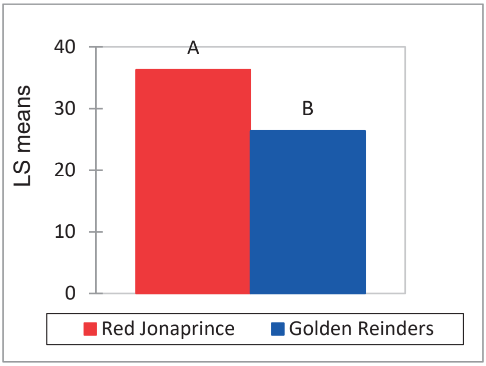

Figure 3. Comparison of shoots length (left-Red Jonaprince/B9, right-Golden Reinders/M9)

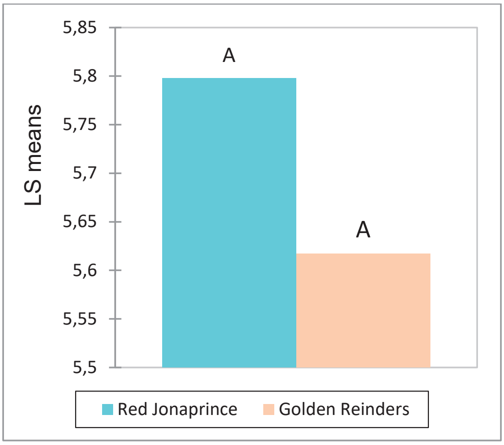

Figure 4. Comparison of TCSA (left-Red Jonaprince/B9, right-Golden Reinders/M9)

Regarding tree height (Figure 5) we can conclude that the studied cultivars gave slight similar growth habit in the first years, 1.46 m was registered at Golden Reinders ®/M9 and 1.43 m at Wilton`s Star Red Jonaprince Select ECO®/B9.

The numerical differences between the variants were multifactorial, mainly due to rootstock and scion influence.

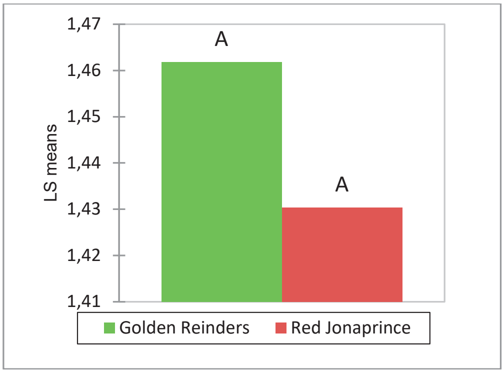

Figure 5 Comparison of tree height (left-Golden Reinders/M9, right-Red Jonaprince/B9)

The highest **generative** bud ratio (Figure 6) was found at GR (31% from total) versus WRJSE (21% from total), highest crop load (Table 2) was found on GR (3.53 fruit/cm^2) in comparison with WRJSE (1.67 fruit/cm^2), while the highest number of fruit per tree (Figure 7) was found in GR (16 in GR and 8 in WRJSE).

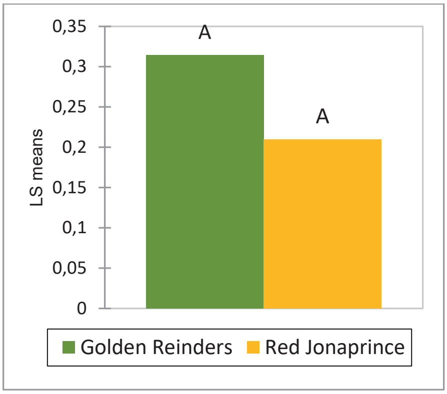

Figure 6. Comparison of generative buds (left-Golden Reinders/M9, right Red Jonaprince/B9)

Table 2. Crop load in treatment cultivars

Cultivars	Crop load (no fruit/cm^2)
Golden Reinders/M9	3.532 a
Red Jonaprince/B9	1.669 b

The results regarding the biometric measurements showed that the size and diameter of fruits (Table 3) was specific to the varieties, with the largest fruit in for Wilton`s Star Red Jonaprince Select ECO®/B9 cultivar with 69.76 mm, followed by Golden Reinders ®/M9 with 62.05 mm.

Table 3. Average fruit diameter of treament cultivars

Cultivars	Fruit Diameter (mm)
Red Jonaprince	69.757 a
Golden Reinders	62.054 b

The indicator number of fruits per tree (Figure 7) showed that at Golden Reinders/M9 there were registered an average of 15 fruits/tree and 7 fruits per tree at Wilton`s Star Red Jonaprince Select ECO®/B9. Golden Reinders/M9 in our researches reached double the value obtained by WSRJ showing the extraordinary bearing capacity of this scion-rootstock combination, but the size of fruits was different.

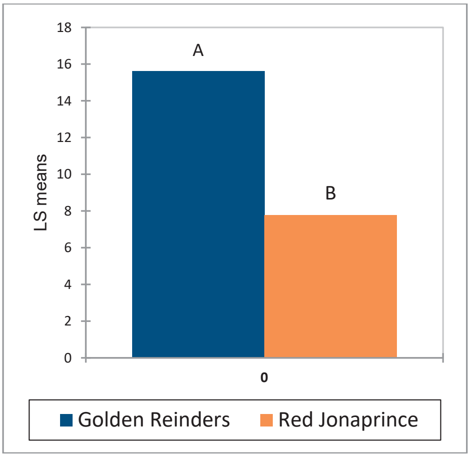

Figure 7. Comparison of number of fruits per tree (left-Golden Reinders/M9, right-Red Jonaprince/B9)

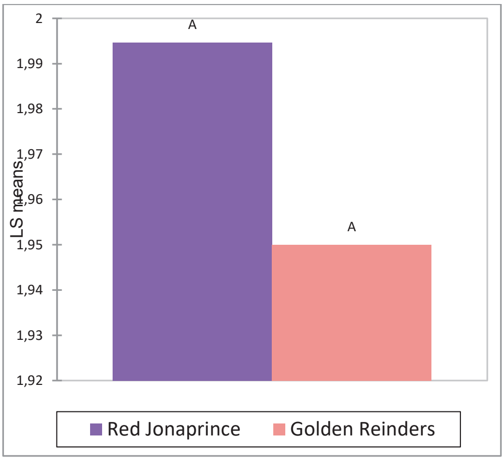

Figure 8. Comparison of the yield kg/per tree (left-Red Jonaprince/B9, right - Golden Reinders/M9)

Results regarding yield (kg/tree) (Figure 8) showed quite similar results in the first years of development, showing 1.99 kg/tree in Wilton`s Star Red Jonaprince Select ECO®/B9 and 1.95 kg/tree in Golden Reinders®/M9.

Brix degree of soluble solids content were ranging between 21-26.4 for WRJSE cultivar and between 12-15.8 for GR cultivar.

The studied cultivars reached very good fruit surface color (80.23-99.9%), especially at Wilton`s Red Jonaprince Select Eco ®/B9.

Csihon et al. (2015; 2022) determinated the fruit surface color and color intensity of the surface for WRJSE fruits, for two consecutive years and the fruit surface color varied from 79 to 83, thus our results are in agreement with our collegues results.

Acording to Racsko et al. (2006), fruit no/tree in GR/M9 varied between 38.2-44.7 fruits/tree showing no extreme differences between on and off alternating bearing years. The smallest number of fruit per tree was 14.8, our results having close values (15.63). Similar results were observed at crop load indicator in GR (6.4 fruit/cm^2) with the lowest values of 1.1 fruit/cm^2, in our results being 3.53 fruit/cm^2.

Average fruit diameter in our results (62.05 mm) had close values to those obtained by Racsko et al.(2006) with values of 79.9- 84.9 mm.

Yield data showed that the trees registered 3.5-4.5 kg/tree in the experiments of Racsko et al. (2006) in the off year, with quite greater results than ours, due to the 4th year of age of trees.

Regarding TCSA, our results (5.61 cm^2) were similar to Csihon et al. (2015) results, which conclude that for WRJSE/M9 the trunk cross-sectional area was 8.2 cm^2. Our scion/rootstock combination WRJSE/B9 registered a weaker growth because the vigor of B9 was slightly weaker than M9, thus the slightly weaker growing habit influences the weaker growth in TCSA.

CONCLUSIONS

Results showed that the planted trees obtained by Knipp technology produced fruits from the first years after establishment, thus the bearing capacity of both cultivars in drip irrigation conditions was relatively good. The fruits obtained at Wilton`s Star Red Jonaprince Select ECO®/B9 rootstock combination achieved a good diameter for marketing and at Golden Reinders®/M9 the bearing capacity is extraordinary with many fruits per tree which showed the necessity of thinning. Further research will be made in order to study the proper adaptation of the studied scion-rootstock combinations in molic eutricambosoils in Northern Transylvania climate region.

Our study provided useful information regarding the expectancy of vegetative and generative development characteristics of the studied cultivars in the first years after establishment, for fruit growing specialists and farmers for establishment of new plantations in molic eutricambosoils.

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GENETIC VARIABILITY STUDY OF SEVERAL ROMANIAN BLUEBERRY CULTIVARS USING ISSR MOLECULAR MARKERS

Cosmin Alexandru MIHAI[1], Maria Cristina IONESCU[2], Adrian ASĂNICĂ[1], Dan POPESCU[1], Mihaela IORDĂCHESCU[2]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaela.iordachescu@qlab.usamv.ro

Abstract

High-bush blueberry is an important economic and nutraceutical species, due to its high content of anthocyanins and high antioxidant activity. Nowadays, climate changes are affecting plant growth and development, leading to changes in plants' adaptation to new environmental conditions. Consequently, the need to create new varieties and hybrids to cope with changes and meet the growing demands of consumers is imperative. One way to hasten plant breeding is to employ molecular methods, such as ISSR. In the present study, five ISSR molecular markers were used to study the genetic relationships between seven Romanian blueberry cultivars and one hybrid. The dendrogram obtained following ISSR analysis revealed the presence of two clusters, one cluster containing 'Lax', 'Prod', 'Vital', 'Azur', and the second one 'Simultan', 'Delicia', 'Compact', and the hybrid obtained through free pollination of variety 'Compact'. The shortest genetic distance was noted between the hybrid genotype and the 'Compact' cultivar. The longest genetic distance was noted between the varieties 'Compact' and 'Prod'. The study also revealed common markers for the hybrid studied and its maternal genitor, that could be used as markers in blueberry breeding.

Key words: *Vaccinium corymbosum L., microsatellites, blueberry breeding, genetic variation.*

INTRODUCTION

Blueberries are very much appreciated fruits in today's culture, not only for their taste and flavour, but also for their nutraceutical qualities (Golovinskaia & Wang, 2021).
Consumed fresh or preserved by freezing, drying, or as gems and compotes, cooked in desserts or consumed for breakfast in yogurts, shakes and juices, they are part of people's diet, including those suffering of diseases such as diabetes or high blood pressure (Afrin et al., 2016; Bouyahya et al., 2022; Hameed et al., 2020; Wang et al., 2021). The demand for good looking and pleasant tasting fruits is linked with the growing demand for high fruit content of anthocyanins from the consumer side, and with the demand of resistance to biotic and abiotic stress from the growers' side (Asănică, 2018).
Consequently, the need for new cultivars answering all these demands is of high importance, and it translates into the plant breeders need for a wide range of genetically diverse genitors to be involved in obtaining novel varieties in shorten time.
Molecular biology techniques can supply at least partially the answers to these demands, as molecular markers can assess not only the genetic diversity across the range of genotypes available to plant breeders, but also label certain genes of interest for fruit quality or for cultivar resistance to stress, and hasten the time needed to check if the traits of interest have been transferred to the prospective new varieties (Debnath & An, 2019; Edger et al., 2022; Grover & Sharma, 2016; Iwata et al., 2016; Lobos & Hancock, 2015; Nadeem et al., 2018).
ISSR (Inter simple sequence repeat) technique takes advantage of the presence in the genomes of microsatellites or simple sequence repeats (SSRs). SSRs are regions that consist of short DNA sequences, two to five nucleotides in size, repeated in tandem multiple times (Gupta et al., 1996). The ISSR technique uses a single primer in the PCR reaction, its sequence based on the microsatellite sequence, to amplify the region

between two identical microsatellites. Since the sequences amplified may or may not be conserved, the technique is useful in studies such as genetic diversity assessment and gene mapping (Pradeep Reddy et al., 2002).
Microsatellite molecular markers have already been mined and developed in *V. macrocarpon* Ait. (Schlautman et al., 2015; Xu et al., 2021; Zhu et al., 2012), and have been used to study genetic diversity in various blueberry populations (Bhatt & Debnath, 2021; Rodriguez-Bonilla et al., 2020; Vega-Polo et al., 2020).
Present study analyses the genetic diversity of eight Romanian blueberry genotypes using ISSR markers, as well as the inheritance of genetic material from the maternal genitor 'Compact' to a hybrid obtained through free pollination, demonstrating the utility of the technique to plant breeders.

MATERIALS AND METHODS

Materials

Eight blueberry (*Vaccinium corymbosum* L.) genotypes, seven Romanian cultivars ('Lax', 'Prod', 'Vital', 'Azur', 'Simultan', 'Delicia', and 'Compact') and a hybrid resulted by free pollination of the cultivar 'Compact', were analyzed in the present study.

Genomic DNA extraction

Extraction of genomic DNA from young leaves was performed using the Innu PREP Plant DNA I KIT IPC 16 (Analytik Jena GmbH+Co, Jena, Germany) according to the manufacturer instructions. Frozen tissue was grounded to powder with liquid nitrogen.
Briefly, for each sample, approximately 100 mg of powder was transferred to 1.5 ml tubes, and then 600 µl lysis solution SLS and 20 µl proteinase K were added to the sample. Thereafter, the samples were incubated for 1 hour at 65°C, centrifuged for 5 min at 10000 x g, and then the supernatant was transferred to prefilters fitted to collection tubes. After an additional centrifugation for 2 min at 10000 x g, 2 µl of RNase A (10 mg/ml) were added and samples were incubated for 5 min at room temperature. After external lysis, samples were further processed in the InnuPure C16 (Analitik Jena), using the Ext_Lysis_200_C16_04 program. DNA quality and quantity were checked with Nanodrop 1000 (Biorad).

ISSR reactions

PCR reactions were performed using the Platinum II Hot Start kit (Invitrogen) according to the manufacturer instructions. PCR setup was done according to Table 1.

Table 1. ISSR reaction setup

Component	Volume	Final concentration
Nuclease-free water	0.2 µl	-
Platinum™ II Hot-Start PCR Master Mix (2x)	5 µl	1x
10 µM Primer P59	0.3 µl	0.3 µM
10 ng/µl Template DNA	2.5 µl	2.5 ng/µl
Platinum GC Enhancer	2 µl	-
Total	10 µl	-

Annealing temperature optimization (between 30°C and 35°C) was done for all primers. PCR program consisted of an initial denaturation step of 2 min at 94°C, followed by 35 cycles of denaturation 15 sec at 94°C, annealing 15 sec at 48/51°C (depending on the primer), and extension 1 min at 68°C, and a final extension step of 2 min at 68°C. The nucleotide sequences of the primers used are presented in Table 2.

Table 2. ISSR primers nucleotide sequences

Primers	Nucleotide sequence
ISSR 2	5'- ACACACACACACACACG-3'
ISSR 3	5'- CTCTCTCTCTCTCTCTRC-3'
ISSR 5	5'- CACACACACACACACARG-3'
ISSR 8	5'-GAAGAAGAAGAAGAAGAA-3'
ISSR 9	5'-ATGATGATGATGATGATG-3'

DNA fragments amplified in the ISSR reactions were separated on 1.5% agarose gel, visualized with the Pharox FX system (BioRad, California, USA), and band lengths were measured using the Quantity One software (Version 4.6.9., BioRad, California, USA).

Data analysis

Data were analysed with BIO-R software, Biodiversity Analysis with R for Windows, (version 3.0, BioRad, California, USA). Amplicons were scored as present (1) or absent (0) as a binary matrix in a *.csv file.

The Resolving Power of a primer (Rp) was calculated with the formula $\sum I_b$, $I_b = 1 - [2(0.5 - p_i)]$, where I_b = band informativeness, and p_i is the proportion of the genotypes contain-ning the band (Prevost & Wilkinson, 1999).

RESULTS AND DISCUSSIONS

All primers used proved to be polymorphic, demonstrating on one hand their suitability for this type of analysis, and on the other hand the variability of the genotypes under study. Out of the five ISSR primers used, three are based on dinucleotide repeats (ISSR2, ISSR3, and ISSR5) and two are based on trinucleotide repeats (ISSR 8 and ISSR 9) (Table 2).

Primer ISSR 2 amplified the 1370 bp band only in the ‘Compact’ variety, the 740 bp band only in the ‘Vital’ variety, the 650 bp band only in the ‘Delicia’ variety, and the 460 bp band only in the ‘Azur’ variety. With the primer ISSR 3, the 1490 bp and 430 bp bands were specific for the ‘Lax’ variety, the 1630 bp band was specific for the ‘Prod’ variety, the 1600 bp band was specific for the ‘Vital’ variety, the 340 bp band was specific for the ‘Azur’ variety,

Since one of the genotypes studied is a hybrid obtained by free pollination of ‘Compact’ variety, the amplified fragments with the same sizes for both hybrid and its maternal genitor were noted, as these markers could be inherited from the parent genotype. A total of 14 bands were common between the hybrid studied and its maternal genitor, 4 for ISSR 2 (940 bp, 790 bp, 600 bp, and 520 bp), 3 for ISSR 3 (960 bp, 800 bp, and 540 bp), 1 for ISSR 5 (700 bp), 4 for ISSR 8 (2250 bp, 1300 bp, 680 bp, and 570 bp), and 2 for ISSR 9 (2300 bp and 1700 bp) (Table 3). However, two of the amplified fragments, with lengths of 790 bp amplified with ISSR 2, and 680 bp amplified with ISSR 8, should not be taken into consideration, as they are monomorphic among all genotypes studied. The rest of the common amplified fragments are potentially inherited from the maternal genitor and should be sequenced, and their location identified within the genome, to check if they are within regions that contain genes of interest for resistance to biotic/abiotic stress or genes of interest related to fruit quality and could be used as potential markers to select for/against traits of interest by the plant breeders.

Table 3. The number of polymorphic and monomorphic loci corresponding to each ISSR primer used

Primer	Number of polymorphic loci	Number of monomorphic loci	Amplified fragments sizes (bp)*	Resolving power
ISSR 2	16	1	1370, 1360, 1320, 1080, 1050, 1020, 940, 890, **790**, 740, 670, 650, 620, 600, 520, 500, 460	8.50
ISSR 3	22	0	1630, 1600, 1490, 1440, 1200, 1170, 1150, 1000, 960, 880, 860, 800, 750, 740, 710, 605, 540, 430, 410, 380, 340	11.75
ISSR 5	21	0	2160, 2110, 2060, 1860, 1520, 1570, 1400, 1370, 1340, 1240, 1200, 1180, 1080, 1040, 1000, 750, 720, 700, 600, 570, 550	12.25
ISSR 8	28	1	2570, 2500, 2350, 2250, 2100, 2000, 1840, 1780, 1670, 1590, 1560, 1470, 1300, 1250, 1110, 950, 860, 800, **680**, 640, 580, 570, 560, 420, 410, 300, 290	14.50
ISSR 9	15	0	2440, 2400, 2300, 2180, 1800, 1700, 1610, 1500, 1340, 1050, 800, 770, 540, 530, 500	7.25

*Fragments written with bold letters represent monomorphic loci. Fragments underlined represent common markers for the hybrid studied and its maternal genitor, the ‘Compact’ cultivar.

The genetic variability of the genotypes under study is reflected into the calculated Rogers’ genetic distances visible in Table 4. As expected, the shortest genetic distance was noted between the hybrid genotype and its maternal genitor, the ‘Compact’ cultivar (0.52).

The longest genetic distance was noted between the cultivars ‘Compact’ and ‘Prod’ (0.72). These results are also apparent in the dendrogram presented in Figure 1.
The UPGMA dendrogram is grouping the eight genotypes studied into two clusters, and in each cluster, the genotypes are grouped two by two. In the first cluster, ‘Lax’ is coupled with ‘Prod’, and ‘Vital’ with ‘Azur’, whereas in the second cluster, ‘Simultan’ is coupled with ‘Delicia’ and the hybrid with its maternal genitor, ‘Compact’.

Table 4. Rogers’ genetic distances calculated with the Bio-R software

Cultivar	Lax	Prod	Vital	Azur	Simultan	Delicia	Compact	Hybrid
Lax	0.00	0.64	0.67	0.69	0.65	0.63	0.66	0.57
Prod	0.64	0.00	0.63	0.66	0.69	0.68	0.72	0.62
Vital	0.67	0.63	0.00	0.60	0.65	0.71	0.66	0.68
Azur	0.69	0.66	0.60	0.00	0.61	0.64	0.70	0.61
Simultan	0.65	0.69	0.65	0.61	0.00	0.58	0.59	0.59
Delicia	0.63	0.68	0.71	0.64	0.58	0.00	0.59	0.54
Compact	0.66	0.72	0.66	0.70	0.59	0.59	0.00	0.52
Hybrid	0.57	0.62	0.68	0.61	0.59	0.54	0.52	0.00

The shortest Roger’s genetic distance between varieties is marked with green, and the longest genetic distance is marked with blue.

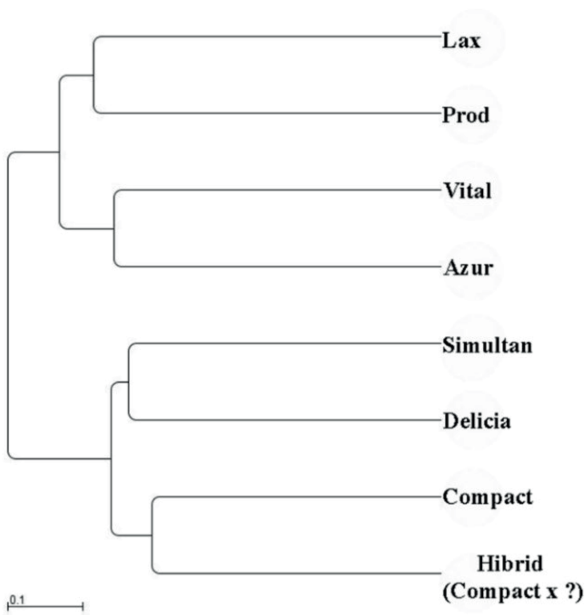

Figure 1. Dendrogram based on the ISSR data generated with the Bio-R software

CONCLUSIONS

Five ISSR molecular markers were used to assess the genetic variability among eight blueberry genotypes, the shortest genetic distance being observed between the hybrid genotype and its maternal genitor, the 'Compact' variety, and the longest genetic distance, observed between the cultivars 'Prod' and 'Compact'.

Twelve amplified DNA fragments were identified in the hybrid under study as regions potentially inherited from the maternal genitor, 'Compact', fragments that should be assessed in a future project for becoming putative markers for traits of interest.

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PERSPECTIVE GENOTYPES FROM *CHAENOMELES* SP. LINDL. FOR FRUIT PRODUCTION

Teodora MIHOVA, Petko MINKOV, Georgi POPSKI, Boryana STEFANOVA, Silvena TODOROVA

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture Troyan, 281 Vasil Levski Str., Troyan, Bulgaria

Corresponding author email: teodora.mihova@gmail.com

Abstract

Japanese quince (Chaenomeles sp. Lindl.) is fruit-medicine plant that is getting attention in the last year in Bulgaria. It has valuable nutritional, dietary and medicinal qualities and deserves to be introduced into culture in Bulgaria. The experiment was conducted in the three-year period (2018-2020) in the collection of RIMSA Troyan, Bulgaria. The reproductive characteristics of the perspective for fruit production genotypes were studied with an average yield higher than 3.5 kg per plant. They were followed also by fruit weight and biochemical compounds. During the period of the study genotype SCH4 was with the highest average yield (6.88 kg), followed by SCH3 (5.09 kg). The thornless genotype SCH 6 is with the lowest yield (3.71 kg).

Key words: *Chaenomeles sp. Lindl., Japanese quince, phenology, fruits, yield, Bulgaria.*

INTRODUCTION

In recent years, there has been an increasing interest in preserving and researching the gene pool of both cultivated and wild species, including those with ornamental and medicinal value. Additionally, there has been a focus on developing products derived from these species. The study of local genotypes enables the identification of species for the purpose of safeguarding, conserving, and enhancing the biological diversity of the local flora (Mezhenskyj, 2004; Minkov, 2012; Stoyanova et al., 2014; Kikindonov et al., 2017; Kizeková et al., 2017). One of these plants is the Japanese quince (*Chaenomeles* sp.), which was distributed in Europe since the end of the IX century. *Chaenomeles* is a perennial plant in the family Rosaceae, subfamily Maloideae, which originates from East Asia. Weber (1963; 1964). had a great deal of credit for the botanical study of this plant species. He identified and described significant taxonomic diversity, including four species and four interspecies hybrids According the current changes of the climate, Japanese quince (*Chaenomeles* sp. Lindl.) is a promising crop due to its increased resistance to cold and drought. In light of environmental pollution and rising population morbidity rates, plants containing biologically active substances with adaptogenic, antimutagenic, immunomodulating, and geroprotective properties are particularly important. *Chaenomeles* sp. Lindl., species and hybrids native to China, Japan, and Tibet, are one such source of valuable substances. Among these, *Ch. japonica* and *Ch. x superba* (Frahm.) Rehd. are the most widespread, thanks to their high resistance to cold and drought. In Japan, Korea and China, it has been grown and used for centuries as a food, medicine, ornamental plant (Mezhenskyj, 2015; Sahin, 2020; Turkiewicz et al., 2020).

The fruits have different shapes, weight and are rich in chemical composition. They are used in the food and pharmaceutical industries (Mezhenskyj, 2009; Rumpunen, 2010; Nahorska et al., 2014; Kaufmane and Ruisa, 2018). Some genotypes fruits, which can weigh up to 150 g which are a valuable raw material for the food processing, pharmaceutical, and perfume industries due to their rich chemical composition. Moreover, Japanese quince can provide raw material for industry quickly and affordably, meeting contemporary ecological production standards. *Chaenomeles* sp. Lindl. is characterized by early onset of fruit-bearing, high yield (up to 2-10 kg per shrub), high ecological plasticity, resistance to pests and diseases, and low production costs (Komar-Temnaya et al., 2001; Rumpunen, 2002; Mezhenskyj et al., 2019). According to Rumpunen and Garansson's research in 2003, the fruits of *Chaenomeles* sp. have excellent qualities and are well-received by consumers in various products such as ice cream, lemonade, jam and ext.

Chaenomeles sp. has been a popular ornamental plant in Bulgaria since the late 19th and early 20th century, as documented by Mondeshka (2005) and Mihova (2016). However, it has only gained popularity as a fruit crop in recent years.

The aim of the present study was to examine the perspective genotypes *Chaenomeles* sp. Lindl. distinguished by a series of valuable economic qualities.

MATERIALS AND METHODS

The study was conducted during 2015-2019 at the RIMSA Troyan collection plantation of *Chaenomeles* sp. to explore the available genetic resources. The study focused on six genotypes from seedlings that were obtained through open pollination and had valuable ornamental, medicinal, and nutritional qualities. The soil preparation for new plantations involved the trench method with local stocking organic fertilization, and the used planting scheme was 2.5 m/1m. The shrubs were grown without irrigation; with natural grassed inter rows that were mowed several times depending on the rainfall during the vegetation period (Table 1). The study examined

The following indicators were studied:

Phenological calendar (according to BBCH scale):

- onset of vegetation;
- onset of flowering;
- full flowering;
- end of flowering;
- picking ripeness of fruit;
- harvest.

Reproductive indicators:

- average fruit weight (g);
- yield per shrub (kg).

In terms of rainfall, the area of Troyan is within the average rainfall for the country, which varies around 410 l/m² for the period from March to August.

The data analyses were conducted by Lidanski (1998) and programs by Microsoft Excel.

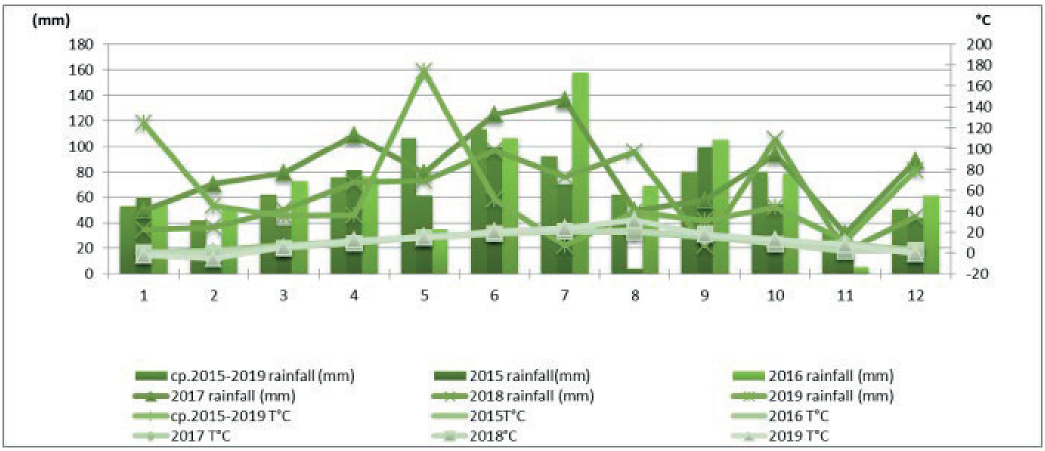


Figure 1. Climatic factors: temperature (°C), precipitation (mm), air humidity (g/m³).

RESULTS AND DISCUSSIONS

Throughout the study period, it was observed that all selected genotypes of *Chaenomeles* sp. initiated their vegetation simultaneously. The vegetation of the six genotypes, began between March 5th to 10th, to the unusually warm weather during the first few months of 2015, which had an average temperature of 7.5°C in March (Table 1). The earliest bud bursting occurred on in genotype SCH5 in 2015, and on February 28th in 2017, while the latest was observed on April 1st in genotype SCH1 in 2012.

Table 1.Phenology of selected genotypes of *Chaenomeles* sp (acording to BBCH scale)

Genotypes	Year	BBCH (01)	BBCH (61)	BBCH (65)	BBCH (69)	BBCH (87)
SCH1	2015	10 .03	15 .04	26 .04	11 .05	15 .10
	2016	18 .03	22 .04	25 .04	15 .05	07 .10
	2017	13 .03	26 .04	29 .04	14 .05	07 .10
	2018	28 .03	19 .04	27 .04	10 .05	03 .10
	2019	01 .04	21 .04	27 .04	03 .05	29 .09
SCH2	2015	10 .03	16 .04	24 .04	12 .05	15 .10
	2016	20 .03	22 .04	25 .04	12 .05	07 .10
	2017	12 .03	23 .04	28..04	15 .05	07 .10
	2018	28 .03	19 .04	27 .04	10 .05	03 .10
	2019	03 .04	26 .04	29 .04	5 .05	22 .09
SCH3	2015	05 .03	07 .04	20 .04	10 .05	05 .10
	2016	08 .03	14 .04	20 .04	16 .05	07 .10
	2017	08 .03	10 .04	19 .04	17 .05	07 .10
	2018	03 .03	12 .04	18 .04	5 .05	01 .10
	2019	27 .03	19 .04	27 .04	10 .05	27 .09
S4	2015	09 .03	15 .04	22 .04	10 .05	17 .10
	2016	18 .03	20 .04	24 .04	11 .05	26 .10
	2017	09 .03	26 .04	29 .04	13 .05	26 .10
	2018	26 .03	14 .04	25 .04	10 .05	29 .10
	2019	27 .03	18 .04	25 .04	03 .05	01 .10
SCH5	2015	16 .02	06 .04	16 .04	10 .05	15 .10
	2016	05 .03	10 .04	22 .04	11 .05	20 .10
	2017	28 .02	03 .04	18 .04	11 .05	20 .10
	2018	15 .03	10 .04	19 .04	06 .05	29 .09
	2019	20 .03	11 .04	24 .04	03 .05	01 .10
SCH6	2015	10 .03	13 .04	24 .04	10 .05	29 .10
	2016	13 .03	20 .04	23 .04	12 .05	10 .10
	2017	11 .03	13 .04	19 .04	13 .05	10 .10
	2018	27 .03	22 .04	28 .04	10 .05	03 .10
	2019	29 .03	16 .04	25 .04	02 .05	24 .09

The onset of vegetation varied within wider limits in 2016 and 2017. Genotype SCH5 showed the earliest onset of vegetation, ranging from 28th February (2017) to 5th March (2016), while genotype SCH2 showed the latest onset, on 20th March in 2016. In the last two years of the experiment, all genotypes except SCH5 entered into vegetation at the same time, towards the end of March and on 1st April (Table 1).

Genotype SCH5 was found to be an early flowering genotype as it began to bloom first in all five years of the experiment, with flowering starting by 11th April. Genotype SCH3 followed, with flowering beginning in the second ten days of April, except in 2015 (Figure 2).

The results of the study show that the onset of vegetation and flowering, as well as the yield of fruits, vary among different genotypes of *Chaenomeles* sp. The earliest bud bursting and flowering was observed in genotype SCH5, which also had the highest yield of fruits in 2016. Genotype SCH4 also showed high yield in 2016, which could be due to the high precipitation in September of that year.

Flowering started in the second half of April in all years of the experiment in genotypes SCH1, SCH2, SCH4 and SCH6 with few exceptions.

The period of full flowering from the initial began three days after the opening of the first blooms in genotype SCH1 (in 2010); SCH2 (in 2009 and 2012); SCH4 (in 2010) and SCH6 (in 2010) to thirty days in genotype SCH3 in 2008 and SCH5 in 2012. Only in genotype SCH5 in 2009 the full flowering started 16 days after the onset of flowering.

Fruits from genotypes SCH1, SCH2 and SCH3 reached picking ripeness in the first ten days of October in 2008, 2009, 2010 and 2011. They ripened at the end of September only in 2012 because in July and August that year the average month temperature reached the record-breaking values of 24.2ºC and 32.3ºC, respectively. In the rest three genotypes - SCH4, SCH5 and SCH6 fruits were harvested 3-4 weeks later.

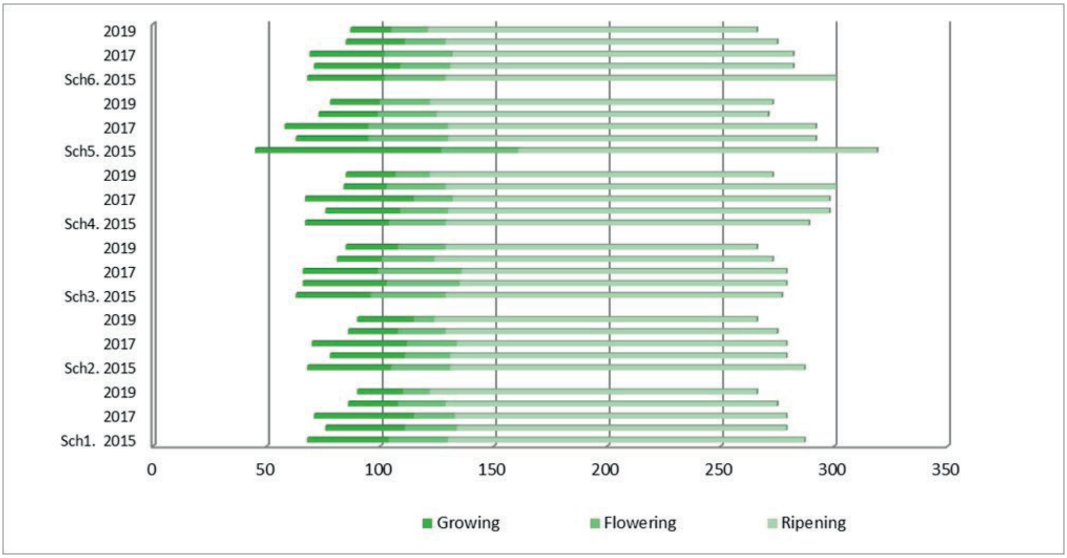
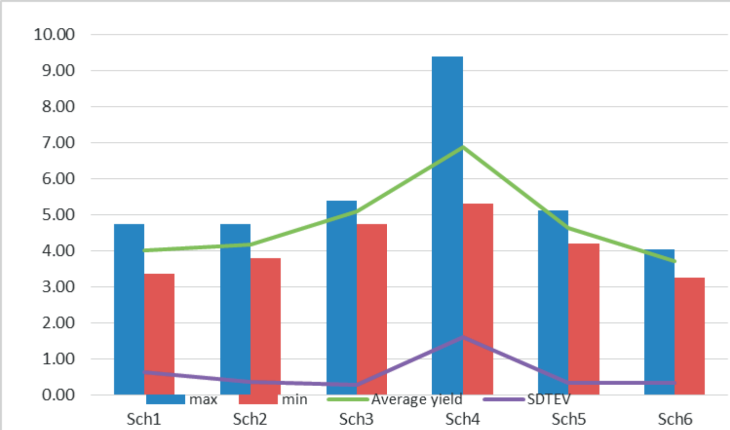


Figure 2. Phenogram of selected genotypes of *Chaenomeles* sp.

Figure 3. Weight of fruits of the studied genotypes of *Chaenomeles* sp. (g)

The study also notes the effect of climate factors, such as temperature and precipitation, on the growth and yield of *Chaenomeles* sp. For example, the warm weather in March 2015 resulted in earlier onset of vegetation and flowering, while the high temperatures in July and August 2012 resulted in earlier ripening of fruits. The study provides valuable information for breeders and growers of *Chaenomeles* sp. in selecting and managing genotypes for optimal growth and yield under different environmental conditions.



Analysis of variance (ANOVA): LSD 0.05 - 2.57 ;LSD 0.01 - 3.46; LSD 0.0 01 - 4.58

Figure 4. Yield from a shrub of the studied genotypes of *Chaenomeles* sp. (kg)

The fruits of the investigated genotypes of Japanese quince exhibit differences in their shape, color, and size. Among the genotypes studied, Sch4 and Sch6 had the highest average fruit weight, both weighing around 60 g. On the other hand, genotypes SCH3 and SCH1 had the medium fruits, weighing 38.08 g and 40.59 g, respectively (Figure 3).

CONCLUSIONS

The studied genotypes of *Chaenomeles* sp. Lindl. demonstrate notable differences in various parameters. There is considerable variation in reproductive characteristics, including fruit weight and yield per shrub. Additionally, there are significant differences in the timing of the phenologycal stages: the onset of vegetation, flowering, and fruit harvest, with a wide interval between genotypes.

Among the studied genotypes, SCH4 and SCH5 stands out as highly productive, while SCH3, and SCH6 are moderately fruitful. Notably, genotype SCH4 appears to be the most promising for cultivation in the Troyan region, based on the study results.

Based on the study results, it can be inferred that the *Chaenomeles* sp. plant can grow and produce fruit successfully in the Central Balkan Mountains region.

Additionally, the research indicate that *Chaenomeles* sp. is relatively undemanding in terms of soil and plant protection requirements.

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HEIRLOOM VALUABLE LOCAL CULTIVARS THREATENED WITH EXTINCTION IN THE CENTRAL BALKAN MOUNTAIN REGION IN BULGARIA

Petko MINKOV, Teodora MIHOVA, Silvena TODOROVA, Georgi POPSKI, Boryana STEFANOVA

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture Troyan, 281 Vasil Levski Str., Troyan, Bulgaria

Corresponding author email: petco_m@abv.bg

Abstract

The study was conducted in the towns of Troyan and Apriltsi and the nearby villages and hamlets in the Central Balkan Mountain. During the expedition research, heirloom local apple cultivars and forms threatened with extinction were discovered, marked and described. Their reproductive characteristics were studied. A large genotypic diversity of the genus Malus, L. have been observed. The complex of agroecological conditions favours the growth, and longevity of the apple trees*. Most discovered and described trees are more than 100 years old, more than 15 m tall and more. The trees bear fruit abundantly, but there is a tendency to alternate their fruit bearing. The subject of the present study are 11 late-ripening valuable cultivars with large-size fruit threatened with extinction, such as 'Kandile' - 129.51 g, 'Sadova Perusha' - 108.07 g, etc., with the aim of studying their morphological, pomological and reproductive characteristics. It has been found that most of them are distinguished by an attractive appearance, intensely coloured fruit skin and valuable nutritional and dietary qualities. Their long-term storage under home conditions is also a valuable quality.*

Key words: *apple, gene pool, local cultivars and forms, biometric, physicochemia of fruit.*

INTRODUCTION

In the recent and distant past, apples were consumed fresh and mostly processed, dried or made into cider, vinegar or applesauce. People have cultivated cultivars specially suited for specific purposes. The fruits range from crispy to floury, from very tart to extremely sweet, from juicy to dry, and each has its best application. If the skin of an apple is tough and waxy, or brown and rough, it keeps well because this prevents the flesh from drying out. Today, some old 'Golden Delicious', 'Jonathan' and 'McIntosh' cultivars are on the market as more widely available, but there is not enough demand for most heirloom apples to make commercial production worthwhile. For example, apples that are red, with spotted skin, rust, or completely covered with a rough texture are not considered pretty, but the taste of many of them is amazing. The bad news is that the number of apple cultivars considered at risk of being lost to the wild and from the food table is greater than for any other type of food. To reverse this trend of loss of diversity in the market for one of our most beloved fruits and ensure that generations from now do not think of 'Red Delicious' (or even the much-hyped 'Honeycrisp') as everything an apple can be in terms of taste, texture, shelf life and use, work must be done to restore apple diversity to our farms, backyard orchards, restaurants and home tables.

Despite historical losses, unique apples are still being found. Historic North American apple (*Malus domestica*) orchards that flourished in the late 19th and early 20th centuries, with cultivars different from today's orchards, are disappearing. There are several reasons for this loss: tree aging, tree maintenance costs, and urbanization. Many groups have compiled local knowledge of apple history and horticulture using both phenotypic and genotypic identification methods, as (1) some characteristics are highly variable with location, tree age, rootstock, etc.; (2) many cultivars are phenotypically similar; (3) there are many cultivar synonyms (i.e., same cultivar but different names) that are not fully realized until genotypically proven; (4) information is missing or scarce for some heirloom cultivars (Wallis et al., 2023).

The interest in preserving the apple diversity requires to find the places where the cultivars remain unique and growing them *in situ* or e*x situ*.

Kiprijanovski et al. (2020), presents the pomological, qualitative and organoleptic properties of 13 autochthonous cultivars, such as: 'Kolačara', 'Ciganka', 'Kojče', 'Crveno pote', 'Pašinka', 'Karapaša', 'Šareno blago', 'Alamanka', 'Pariska palma', 'Djulabija', 'Zvečarka', 'Tetovka' and 'Avajlija', with the cultivar 'Golden Delicious' as reference. Some of them deserve to be grown in traditional orchards in typical rural areas, with reduced use of chemicals and in environmental protection programs. Others of these cultivars do not have much economic and agronomic value, but this local gene pool is of great sociocultural importance and the cultivars should be preserved in *in situ* collections.

Duralija et al. (2021) reported the long tradition of fruit growing in the Republic of Croatia, due to its geographical location, climatic conditions and high quality of fruit crops, there is a growing demand for functional foods obtained from autochthonous and traditional plant sources, as they are recognized as a very valuable source of healthy bioactive ingredients. Heirloom apple cultivars (*Malus domestica* Borkh.) are characterized by good morphological and pomological properties, less need for chemicals during cultivation and a higher proportion of biologically active compounds (BACs). They have better sensory acceptability compared to commercial cultivars. However, their nutritional and biological potential, as well as their ability to be processed into functional food, is underestimated. Conserving heirloom apple cultivars and their cultivation importance through innovative strategies can successfully incorporate them into future selection programs.

Musacchi & Serra (2018) assessed the impacts and potential of both environmental conditions and agronomic factors. Environmental and agronomic factors throughout the vegetation have a strongly impact on the final quality of apples, including nutritional aspects. Temperature and light modify colour quality and dry matter accumulation, but can also cause the unwanted effect of sunburn. Orchard design, shaping, and pruning can dramatically affect fruit skin colour and fruit maturity. Crop load and thinning can determine physiological adjustments that favour fruit dry matter accumulation. Watering and nutrition can change the colour and chemical composition of fruit flesh.

For apple storage, it is known that the later the ripening period, the firmer the fruit flesh and the better and longer the fruit will be stored. Early ripening apples have soft flesh and do not store well. Fruits that are not fully ripe are selected for storage when the seeds are just starting to colour, as for best results temperature and humidity conditions are controlled.

With the spread of commercial apple cultivars since the second half of the last century, hundreds of different local cultivars quickly disappeared from orchards, and the specific quality characteristics of these fruits are still at risk of being lost today. From the 1920s to the 1960s, they were the main part of the apple cultivar type in Bulgaria. As a result of the introduction of new cultivars of American and European selection, their cultivation and distribution is decreasing and they are threatened with almost complete extinction. These cultivars are very well adapted to the agroecological conditions of the Central Balkan Mountain region, the fruits are large-sized with very good taste qualities, good attractive appearance, suitable for long-term storage, relatively resistant to diseases and enemies, which requires them to be sought out, stored and studied. They are suitable for non-conventional orchards, family farms, as well as for selection programs, to improve some of their qualities (Vitkov, 2015; Dzhuvinov et al., 2016).

This genetic diversity is a huge resource that could be used in selection programs that seek high yields, good fruit characteristics, tolerance to diseases and pests, as well as to abiotic environmental factors. To exploit this wealth, efforts should be made to survey different areas to select desirable types for establishing gene stocks (Ercisli et al., 2006).

The objective of the present research is the preservation and study of the valuable local apple cultivars, adapted to the agroecological conditions of the Central Balkan Mountain region, with large-sized fruits, with very good taste qualities, suitable for fresh consumption and processing and resistant to diseases and enemies.

MATERIALS AND METHODS

During the expedition research in the Central Balkan Mountain region, the towns of Troyan and Apriltsi and their adjacent villages and hamlets, at an altitude of 600 to 1100 m, old local apple cultivars threatened with extinction were discovered, marked and described.

The trees are over 100 years old, with a large trunk circumference (over 1.5 m), crown height over 15 m, with good vitality. They are grafted at a height of 30-40 cm from the ground, and some species at 1.20-2.00 m. The shape of the crowns varies from globose to freely growing cone-shaped. The trees bear fruit abundantly, but there is a distinct alternateness. Marked trees are found as a seperate specimen or in abandoned old orchards, along small hamlets, often overgrown with forest vegetation, without application of agrotechnical and pomological measures. Drying is often observed on the trunk and crown.

The following indicators are taken into account:

- Reproductive - fruit weight (g), fruit size (mm) (height and average diameter) and fruit stalk (mm)

The terminal (king) fruit should be excluded from the sample (UPOV 2005) Observations on the fruit should be made on 10 typical fruits taken from a minimum sample of 20 fruits, at the time of ripeness for eating.

- Dry matter - with refractometer RHB-32 with a range of 0.0-32.0% (Brix %)
- Density (firmness) of fruit flesh (kgf/cm^2) - determined with a digital penetrometer FHT-15 (3.5 mm), by measuring both sides of 25 randomly selected fruits. The fruit skin of the measured fruits was removed.
- Pomological characteristics –
  - sensory analysis (taste, aroma)
  - colour coordinates L - colour brightness; +a - red color; -a - green color; +b - yellow color -b - blue colour measured with Color meter CM-200S, reported according to the CIE Lab system of the fruit skin. A/b colour tone quality indicator was calculated.
- A visual assessment was made of the attitude of the observed cultivars to the economically significant diseases, such as apple scab and powdery mildew on leaves and fruits. Low susceptibility based on single spots or absence of spots by the apple scab on leaves and fruit was reported and no powdery mildew symptoms were detected.

RESULTS AND DISCUSSIONS

According to the Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability (UPOV 2005), the determination of fruit size is divided into 9 groups. The cultivars included in the present study are large-sized in the categories from medium (5) ‘Cox’s Orange Pippin’) to very large (8) ‘Bramley’s Seedling’).

The fruits of ‘Red Winter Calvil’ (229.7 g) and ‘Winter Green’ (196.2 g) had the largest size, followed by ‘Bukhavitsa’ and ‘Perusha’ (about 187 g). ‘Garden Feather’ and ‘Ribstone Pippin’ had the smallest fruit (108 g). Accordingly, fruit height was 68 mm in the largest, but the largest average diameter was registered in ‘Red Winter Calvile’ (82 mm), whereas in ‘Winter Green’ it was only 46 mm, which gives an elongated shape to the fruit of this cultivar. ‘Perusha’ (81.4 mm) and ‘Gankovata’ (76.3 mm) had a larger average diameter. According to UPOV (2005), the shape of the fruits of most of the studied cultivars is (7) obloid, for example, ‘Sadova Perusha’, ‘Ribston Pippin’, ‘Red Winter Calville’. The fruit shape of the cultivars ‘Kandile’, ‘Buhavitsa’ is (6) globose, ‘Winter green’ is (2) conic (Table 1).

The length of the fruit stalk is a characteristic pomological indicator for each cultivar. It is significant for the better appearance of the fruit, it gives a better opportunity for exposure to the sun and a longer time remaining on the tree. Apples with a longer stalk are more preferred by traders, as it favours storage capability. The fruits of ‘Winter green’ and ‘Chardachka’ had the longest stalks (20-22 mm) (Table 1).

Table 1. Biometric characteristics of fruits

Cultivar	Fruit weight (g)	Height (mm)	Diameter (mm)	Fruit: general shape	Fruit stalk length (mm)
	X ± St. Dev.	X ± St. Dev.	X ± St. Dev.		X ± St. Dev.
Candle	129.50±27.58	55.15±4.89	48.81±4.13	Globose (6)	13.84±4.41
Sadova perusha	108.07±29.02	53.46±6.08	64.48±4.81	Obloid (7)	9.87±2.58
Buhavica	185.79±29.50	63.90±2.49	76.62±3.03	Globose (6)	18.59±4.13
Perusha	187.08±37.61	62.82±4.38	81.42±5.58	Obloid (7)	9.10±1.19
Ribstone Pepin	108.45±12.3	54.51±4.44	63.34±1.78	Obloid (7)	15.77±1.19
Red Winter Calville	229.71±73.94	67.76±6.62	82.24±7.20	Obloid (7)	16.74±5.30
Winter green	196.19±10.50	68.03±2.07	46.16±0.87	Conic (2)	22.01±6.60
Chardachka	112.53±11.34	54.86±2.52	70.22±2.69	Conic (2)	19.73±2.92
Gankovata	145.83±4.73	59.05±0.83	76.36±0.23	Obloid (7)	15.61±2.35
Chichovata	115.79±19.40	53.16±4.03	67.42±3.54	Globose (6)	12.59±4.72
Tsar Alexander	153.34±42.08	56.35±5.90	72.92±6.49	Globose (6)	15.92±3.76
LSD 0.05	*43.67*	*5.86*	*5.66*		*5.10*
LSD 0.001	*76.74*	*10.3*	*9.94*		*8.96*

A main characteristic of the sensory analysis is the colour of the fruit skin. The cultivars, such as ‘Kandile’, ‘Perusha’, ‘Winter green’ and ‘Chichovata’ have a green main colour, some of them with white dots (‘Perusha’ and ‘Winter green’), and others with a blush on the exposed side (‘Red Winter’ ‘Calvil’ and ‘Gankovata’), as ‘Perusha’ is with more pronounced rusts.

The cultivars ‘Buhavitsa’, ‘Ribston Pippin’ and ‘Chardachka’ have a red main colour (Table 2). Skin colour is fundamental in apple production as a grading criterion. Many of the most cultivated apple cultivars are two-tone skin color, with a varying range of intensity and quality of red rind colour. Most of the world's apple production is based on mutations of original cultivars such as ‘Gala’, ‘Delicious’ or ‘Fuji’ (Musacchi & Serra, 2018). In automated fruit sorting, machines are usually set to sort based on the percentage of red colouring on the surface of the fruit, such as 'Modi®' and 'Demi Rouge™', with more than 70% dense red and 40 to 70% shades of over colour. For some cultivars, such as ‘Granny Smith’ and ‘Golden Delicious’, excessive red colour is an undesirable quality for the market and highly red apples decrease their commercial value (Musacchi & Serra, 2018).

The fruit flesh is usually white (1 ‘Akane’, ‘Spartan’) - ‘Kandile’, ‘Perusha’, ‘Chardachka’, ‘Tsar Alexander’; in the rest it is greenish (4 ‘Gloster’, ‘Granny Smith’) and rarely cream-coloured (2 ‘Jonagold’) (UPOV 2005), which is characteristic of the newer selected cultivars. The fruit flesh of ‘Kandile’, ‘Buhavitsa’, ‘Ribston Pippin’, ‘Chichovata’ is dense, whereas the rest of the cultivars are soft. The fruits of the observed cultivars are distinguished by juicy flesh. It is rather acidic in ‘Sadova perusha’ and ‘Winter green’ to sweet in ‘Chardachka’ and ‘Chichova’.

The cultivars, such as ‘Kandile’, ‘Tsar Aleksandar’, ‘Chichovata’ have a pronounced intense aroma, whereas ‘Buhavitsa’, ‘Perusha’, ‘Ribston Pippin’ are without aroma.

The researched cultivars satisfy both tastes of both types of consumers of acidic and sweet fruits.

Table 2. Pomological description of the cultivars

Cultivar	Colouring of fruit, taste qualities
Kandile	Fruit skin: green with blush and red non-broken vertical strikes, thick. Fruit flesh: white, juicy, grainy structure, dense, slightly acid without a pronounced aroma, refreshing.
Sadova perusha	Fruit skin: green with blush, dense, thick. Fruit flesh: greenish, soft, juicy, rather acid, without aroma.
Buhavica	Fruit skin: red broken and non-broken darker stipes, with waxing coat, dense, tough, not very thick. Fruit flesh: grenish, crispy, dense, comparatively tender with crystalline structure, juicy, slightly acid, without aroma.
Perusha	Fruit skin: yellowish-green with grey spots and rust around the stalk, thick with wax coating. Fruit flesh: white, soft, slightly floury, dry, sweet, without aroma.
Ribston Pippin	Fruit skin: red with broken vertical darker stripes with white spots and a green cloud around the bottom part. Thick, tough, dense. Fruit flesh: greenish, dense, slightly juicy, slightly fibre-like, firm, rather acid without aroma.
Red Winter Calville	Fruit skin: yellowish-green with an intense red over color and darker non-broken vertical red stripes, sprinkled with white dots, relatively thick, dense with a light wax coating. Fruit flesh: yellowish-green, juicy, crispy, slightly acid, with very good taste.
Winter green	Fruit skin: green with white spots, thick, covered with a wax coating. Fruit flesh: green with grain structure, juicy, rather acid but not tough.
Chardachka	Fruit skin: red in non-broken stripes with white spots, glossy, thin, tough. Fruit flesh: white, soft, slightly floury, dry, sweet, without aroma.
Gankovata	Fruit skin: yellow with a blush on the sunlit side, thin, dense, tough. Fruit flesh: white, soft, slightly mealy, juicy, sweet with an interesting aroma.
Chichovata	Fruit skin: green with red clouds and darker red non-broken stripes, thick, firm. Fruit flesh: green, dense, slightly tough, juicy, sweet with aroma.
Tsar Alexander	Fruit skin: red with dark red broken stripes and yellowish colouring on the dark side. Fruit flesh: white, soft, tender, fine, juicy, aromatic, slightly acid.

In the present study, the colour coordinates L - colour brightness were measured; +a - red colour; -a - green colour; +b - yellow colour; -b - blue colour (Table 3).

Table 3. Fruit colour parameters

	L	a	b	a/b
Kandile	57.73	9.14	40.88	0.32
Sadova perusha	71.74	-6.09	63.42	7.92
Buhavica	57.58	11.92	51.44	0.28
Perusha	74.68	1.95	119.22	*0.02*
Ribston Pippin	52.14	18.60	52.48	0.43
Red Winter Calville	56.79	11.38	57.49	0.24
Winter green	66.26	-1.61	79.88	-0.02
Chardachka	52.27	14.34	48.56	0.34
Gankovata	62.55	0.78	62.85	0.01
Chichovata	62.36	-0.48	44.88	0.04
Tsar Alexander	66.08	2.43	39.27	0.11
LSD 0.05	*11.70*	*11.93*	*14.04*	
LSD 0.01	*15.62*	*19.03*	*16.04*	

Regarding the brightness of the skin, the highest value was recorded for the fruits of ‘Perusha’ (L = 74.68), in which the yellow colour (b = 119.22) was the most dominant. High brightness values are also found in ‘Sadova perusha’ (L = 71.74), Winter green and ‘Tsar Alexander’ (L = 66), and with the lowest value L = 52.14 are the fruits of ‘Ribston Pippin’, in which the red color (a = 18.60) is the most pronounced. The ‘Sadova perusha’ cultivar had the largest negative value (-a = 6.09), defining the ‘Green colour’.

The qualitative indicator colour tone a/b (dominant wavelength) had the highest value for the fruits of 'Sadova perusha' (7.92).
Soluble dry matter (Brix%) was recorded in two periods - after fruit harvesting and 10 days after storage in uncontrolled conditions. The highest values (16.8 and 17% were recorded for 'Perusha' and 'Gankova', as after their storage they also had the highest values, but the change is very small (0.5% to 1%), compared to cultivars that have increased the dry matter after storage to more than 3%. These are 'Winter green', 'Chichovata' and 'Kandile' (Figure 1).
According to Hoehn (2003), soluble solids content, firmness, and acidity are important factors determining the nutritional quality of apples. Tests with consumers have confirmed that acceptance can be predicted by instrumental measurements of total soluble solids (refractometer). 'Golden Delicious' of acceptable eating quality should reach a minimum of 12° Brix for total soluble solids.
The highest values for fruit firmness were measured in the 'Chichovata' with 14.44 (kgf/cm^2) and 'Gankovata' with 13.48 (kgf/cm^2) varieties (Figure 1), which corresponds to the sensory analysis according to Table 2. An intermediate position is occupied by 'Red Winter Calville', 'Chardachka' and 'Ribston Pippin', as with the smallest value for fruit firmness are 'Kandile' and 'Sadova perusha'.
High dry matter cultivars also have firmer fruit, except for 'Kandile', which has 17% dry matter and a firmness of 7.62 kgf/cm^2.
Softer fruits have a lower percentage of soluble solids. Based on this density, cultivars are defined as transportable and suitable for longer term storage.
The importance of combining instrumental methods (objective) with sensory methods (subjective) in the assessment of fruit quality, gives reliable and efficient information about how they are perceived by the consumer. Harker et al. (2002) considered that consumer acceptance of 'Gala' and 'Elstar' appeared to be less dependent on firmness, soluble solids content and acidity, but dependent on flavour quality and juiciness. In this regard, future storage optimization should take into account aroma aspects.
Tests with consumers have shown that liking can be predicted by instrumental measurements of total soluble solids (refractometer), titratable acidity and hardness. 'Golden Delicious' can be accepted if it reaches a minimum of 12° Brix, a minimum acidity of 3.2 g/l and a minimum hardness reading of 44 N. For 'Elstar', hardness should exceed 46 N and soluble solids should be above 12° Brix. 'Elstar' with acidity below 4.0 g/l or high acidity (> 6.5 g/l) did not appeal to consumers (Hoehn et al., 2003).

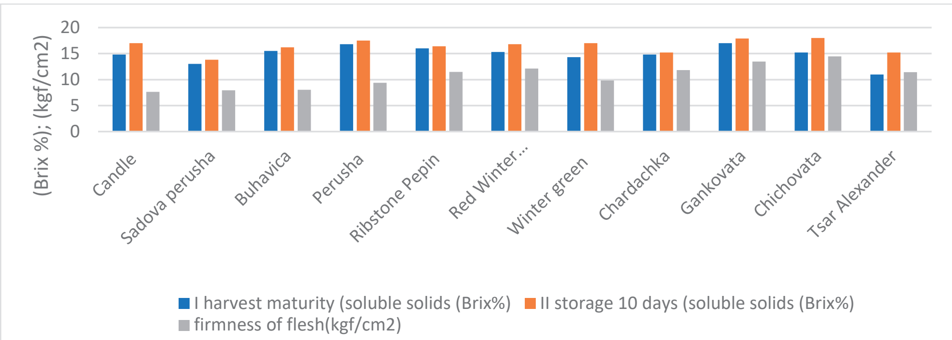

Figure 1. Soluble dry matter (Brix %) and fruit flesh firmness (kgf/cm^2)

A visual assessment was made of the attitude of the observed cultivars to the economically significant diseases, such as apple scab and powdery mildew on leaves and fruits. Low susceptibility based on single spots or absence of scab spots on leaves and fruit was reported and no powdery mildew symptoms were detected.

CONCLUSIONS

Based on the results of the present study, it was found that the 11 apple cultivars studied in terms of their fruit quality characteristics (size, weight, flesh firmness, appearance, taste, etc.) were not inferior to the standard 'Golden Delicious' and 'Granny Smith' cultivars.

The largest are the fruits of the 'Red Winter Calvil' (229.7 g) and 'Winter Green' (196.2 g), the shape of the fruit of most cultivars is obloid, the main colour of the fruit skin varies from green, yellowish-green to red. The fruit flesh is mainly white and green and their shades, some varieties have a pronounced aroma, others are without aroma.

The highest values of dry matter with 16.8 and 17% were reported in the fruits of the 'Perusha' and 'Gankova' cultivars, during ripening stage and after storage for 10 days, and for fruit firmness in the 'Chichovata' with 14.44 (kgf/cm^2) and 'Gankova' with 13.48 (kgf/cm^2).

The 'Winter green' cultivar could successfully replace the most common, cultivated and preferred 'Granny Smith' apple, in small family farms for sustainable and organic farming and non-conventional plantations.

We recommend the cultivarss we are considering to complement the apple assortment under the conditions of the Pre-Balkan Mountain. Their inclusion in selection programs can influence their biological potential and deservedly gain a wider distribution.

We identified promising cultivars best suited for fresh consumption and commercial cultivation, such as 'Tsar Alexander', 'Winter Green', 'Red Winter Calvil'. The rest are suitable both for fresh consumption and for processing (drying, sweet, distillates), and all cultivars of the group are subject to long-term storage, with the exception of 'Tsar Alexander'.

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INFLUENCE OF INTERCROPPING ON PHYSICO-CHEMICAL AND BIOLOGICAL SOIL PROPERTIES IN ORGANIC STRAWBERRY CROP

Andrei MOȚ[1], Mihai FRÎNCU[1], Ioan VLAD[2], Elena Ștefania IVAN[1], Oana-Crina BUJOR[1], Bogdan MIHALCEA[3], Maria-Mihaela ZUGRAVU[1], Liliana Aurelia BĂDULESCU[1, 2], Roxana CICEOI[1]

[1]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania
[2]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania
[3]Cooperativa Agricola Rodagria Produce, Ogoru, Str. Constructia C1, Călărași, Romania

Corresponding author email: oana.bujor@ qlab.usamv.ro

Abstract

The intercropping system promotes biological interactions, increasing plant resources efficiency such as nutrients, water and light use. The study was carried out in polytunnels using the organic Amandine® strawberry variety in the Baragan plain, in the southeast of Romania. Along the polytunnel's pole lines, Borago officinalis L. flower strip was seeded. For soil sampling were chosen two moments (April and October 2022) and were analysed the following physicochemical and microbiological parameters: pH, electrical conductivity, total soluble salts, total carbon and nitrogen and total number of bacteria and fungi. The results pointed out that the number of bacteria in the soil covered with flower strips increased significantly during the vegetation period for borage strips. In addition, Borage proved to be a companion species that inhibit weeds growth successfully, including in high infestation of Johnsongrass. After the first year, no significant differences regarding the soil physicochemical properties between the sampling periods were noticed.

Key words*: flower strip, intercropping, soil health, strawberries, Borago officinalis L.*

INTRODUCTION

The strawberry (*Fragaria x ananassa* Duch.) is a fruit species cultivated worldwide, their fruits being highly appreciated not only for the unique taste but also for the height content in bioactive ingredients and nutrients, and health benefits (Dane et al., 2019; Giampieri et al., 2012; Tulipani et al., 2008; Yang et al., 2020; Mițoi et al., 2022). At the same time, the number of consumers looking for nutritious food and pesticide-residue-free or organically produced berries is increasing. As a result of the need to combat climate change and the demand for wholesome products, it is imperative for farmers to discover effective methods to create innovative and resilient agricultural strategies. These strategies should be focused on maintaining berry production while simultaneously minimizing detrimental environmental impacts and promoting the enrichment of functional biodiversity. In particular, it is known that along with global warming, extreme effects appeared and damaged the habitat both above and below the ground. Moreover, through monoculture technologies, pest control and intensive fertilisation, biodiversity has been greatly diminished (Geisen et al., 2019).

Intercropping is an agricultural practice that involves growing two or more crops in proximity. This method is part of polyculture farming and is used for several reasons, including to increase biodiversity, enhance productivity, make efficient use of resources. decreasing disease transmission, increasing soil quality, crop yield, land efficiency and above all it has an essential role in organic agriculture (Blessing et al., 2022). Organic farming is a growing sector in agriculture that uses fertilizers of organic origin such as compost manure, green manure, and bone meal and places emphasis on techniques such as crop rotation and companion planting or intercropping, with the aim to reduces carbon emissions, improves soil health, and reloads natural ecosystems for cleaner water and air (Mihalache et al., 2015; Gamage et al., 2023).

Accordingly, studies regarding the agrochemical quality of the soil, have shown that intercropping can reduce the leaching of some nutrients from the soil and increase their absorption in the current or future crop (Mocanu et. al., 2016; Moghbeli et al., 2019; Astiko et al., 2021; Romaneckas et al., 2020). Many studies have also noted an increase in soil organic carbon and total nitrogen, depending on the plants used as companions. In particular, clover has proven to be beneficial for maintaining a high level of nitrogen in the soil. A study carried out in 2021 by Li et al. demonstrated the effectiveness of nitrogen absorption by the main crop, intercropped with clover. Other studies that tested intercropping between strawberries and perennial plants showed benefits in terms of weed suppression, water use efficiency, soil biological activity (Dane et al., 2016; Laugale et al., 2023). Another study also demonstrated significant changes on soil biological properties after a period of 3 years of intercropping (Dane et al., 2017).

Intercropping usually leads to a pH decrease, but it depends on the plants used for intercropping (Lian et al., 2019; Romaneckas et al., 2020). Considering that strawberries prefer a moderately to slightly acidic soil (Milosevic et al., 2009), intercropping is a good way to improve soil pH for this crop. Also, the slightly acidic pH solubilizes certain micronutrients, improving their absorption (Ritchey et al., 2014). In addition to phosphorus, potassium, magnesium, strawberries are a great source of iron, zinc, manganese, i.e. the elements that are accessible at a slightly acidic pH (Tulipani, 2012).

At a microbiological level, the soil experiences more pronounced changes than chemical alterations. Research has highlighted substantial variances between monocultures and intercrops, especially when evaluating biodiversity indicators like microbial and enzyme activities, along with the total microbial population (Gong et al., 2019; Lu et al., 2023). For strawberry crops, monocropping is one of the biggest problems, because it generally requires protected spaces that cannot be changed easily. Relevant studies showed a major decrease of soil bacterial community after several years of monocropping (Li et al., 2018; Lovaisa et al., 2017). Using companion plants in open or protected spaces proved to be beneficial for strawberry crop. Companion planting strawberries with borage led to a notable rise in yield and market quality of the strawberries, indicating enhanced insect pollination per plant. The strawberries grown alongside borage showed an average increase of 35% in fruit production and a 32% increase in yield by weight (Boeckmann, 2023; Griffiths-Lee et al., 2020; Kellogg Garden Products, 2020).

This study's objective was to assess the impact of intercropping strawberries with *Borago officinalis* L. in high tunnels, focusing on the physico-chemical properties of the soil and the composition of soil bacterial and fungal communities.

MATERIALS AND METHODS

The experiment was conducted in 2022 at Cooperativa Agricola Rodagria Produce, an organic farm located in Calarasi County, in the south-east of Romania.

Strawberry plants (cv. 'Amandine') were planted in April, in high polytunnels, with 5 raised double-rows per tunnel. *B. officinalis* was intercropped with strawberry being sown in May 2022 along the poles lines of the tunnel, next to strawberry rows, in 60 cm wide strips.

The soil samples were collected in April 2022 (untreated/control) before planting strawberry runners (stolons) and sowing the *B. officinalis* as well as in October 2022. Each sample was homogenized on the spot and was divided into separate containers for physico-chemical and microbiological analyses. The samples were transported to the laboratory in cool boxes (3 hours from the sampling). The soil samples were analysed for the physico-chemical and microbiological parameters: pH, electrical conductivity, total carbon and nitrogen and total number of bacteria and fungi.

Physico-chemical analyses of soil samples

For the physico-chemical analyses, the samples were dried at room temperature for 3 days, after which they were ground in a soil mill (Humboldt, USA) to a size of 2 mm.

The pH of the soil was determined potentiometrically in aqueous extract (SR ISO 10390:2015) and the electrical conductivity

(EC) of the soil was determined conductometrically in aqueous extract (SR ISO 11265:1994), using a multiparameter analyzer (Mettler Toledo Seven Excelence.
The total content of soluble salts was calculated conductometrically, based on EC value (Ilie and Mihalache, 2013).
For the analysis of total nitrogen and total carbon, the soil samples were sieved through a 250 µm sieve (Haver & Boeck, ISO 3310-1:200, Germany). The analysis was carried out by dry combustion method (Moț et al., 2022), using the CHNS elemental analyzer (Elemental Analyzer EA 3100, Eurovector, Italy). All analyzes were performed in triplicate.

Microbiological analyses of soil samples
For the microbiological analyses, the samples were analyzed within 24 hours of collection.
The total number of bacteria (TNB) and the total number of fungi (TNF) was determined by the serial dilution method on PDA medium (Mwangi, 2023; Martin et al., 2012). As a method of determination, the number of colonies were calculated based on colony counting at 1, 3, 5 and 7 days after inoculation, taking into account the dilution and soil moisture (Brugger et al., 2012). All analyzes were performed in triplicate.
Statistical analysis
The statistical processing of the results was carried out using the statistical functions of Excel - Office 2010 program (Pomohaci and Vâșca, 2017). Also, Duncan test (IBM SPSS - statistical analysis software) was used for univariate variance analysis at the $P \leq 0.05$ level to determine the significance between all groups of the variants used in experiments.

RESULTS AND DISCUSSIONS

B. officinalis strip, as it showed very good resistance to competition from weeds in the area. Being a plant with rich foliage, with a relatively well-developed root system, with fairly fast growth and adapted to any type of soil, that is, a plant with a special ecological plasticity that immediately adapted to the respective conditions.
It has been observed that the influence of companion plants is very complex and determines a multitude of effects on some parameters, which in many situations do not have a typical or predictable evolution.
Although some studies did not observe significant changes in chemical parameters (Suri et al., 2019; Gikonyo et al., 2022), our results are rather similar to those of Mansaray et al., (2023) and Romaneckas et al., (2020).
As in the studies mentioned above, it is observed that the pH of the soil had a tendency to decrease in the case of the use of companion plants. At the beginning of the experiment, the soil reaction was neutral. In the case of the control variant (monoculture), an increase in the pH value was observed at the end of the experiment. In the variant of intercropping with *B. officinalis*, a significant decrease of pH by 9.09% was observed (Figure 1).

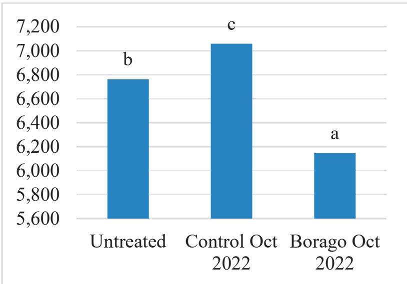

Figure 1. pH evolution from the beginning to the end of the experiment

Regarding soil EC and TSS, a significant decrease is observed from spring to autumn. This decrease is due to the fact that the monitoring areas are located on the edge of the tunnel, in the place where the rainwater drains from the surface of the entire tunnel. Due to this fact, the soil was repeatedly subjected to leaching processes, and the mineral content decreased (Figure 2).

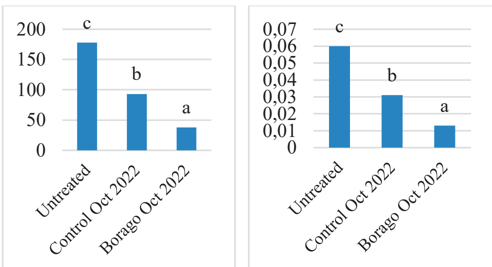

Figure 2. EC and TSS evolution from the beginning to the end of the experiment

It is a known fact that intercropping enhances C and N sequestration, thus improving soil fertility but also contributing to reducing greenhouse gas emissions (Cong et al., 2014; Chen et al., 2023). Soil total nitrogen had a significant increase when *Borago* was used for intercroping, compared to the control (monocropping). It can be noted that on the control version the content was similar both in spring and autumn, without major changes. On the control variant, there was a significant decrease of total carbon, which means a decrease of organic matter and implicitly, a decrease of microorganisms number (Figure 3).

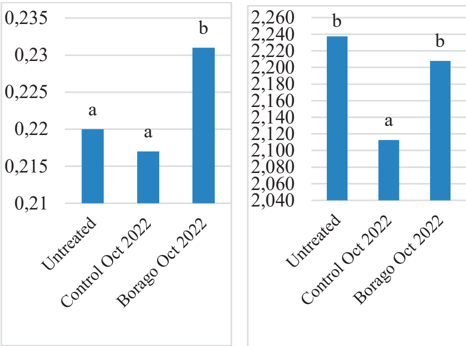

Figure 3. Total nitrogen (N_{tot}, %) and total carbon (C_{tot}, %) evolution from the beginning to the end of the experiment

Although from a statistical point of view the C:N ratio had a significant decrease from spring to autumn, from 10.17 to 9.74 (untreated) and to 9.56 (*B. officinalis* intercropping), from an agrochemical point of view the value remained within the limits of a normal soil (Swangjang, 2015) which means that the microbiological balance has not changed. The fact that both carbon and nitrogen had higher values means that the soil provided more nutrients, which causes a significant increase in microbial activity (Figure 4).

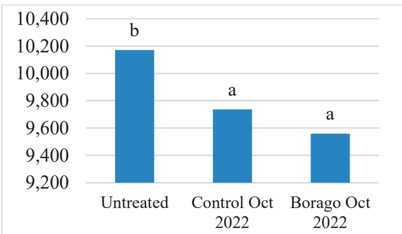

Figure 4. C: N ratio evolution from the beginning to the end of the experiment

Microbiological analyses showed an increase in the number of bacterial colony-forming units (CFU) from April to October 2022, from 15 $*10^6$ CFU/g soil to 1689.33 $*10^6$ CFU/g soil for the control variant, as it included plant species already adapted to specific environmental conditions. In the case of the *Borago* flower strips it was observed an increase in the number of CFU from $15*10^6$ CFU/g soil to 245.66 $*10^6$ CFU/g soil, which indicates the beneficial effect for the organic-maintained soil in the strawberry plantation (Figure 5).

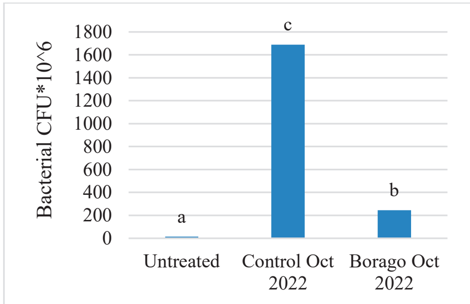

Figure 5. Bacterial CFU evolution in soil samples

In the case of the fungal community in the organic soil in the flowering strips of the strawberry plantation, an increase from 0.33 *106 UFC/g soil to 2 *106 UFC/g soil for the control variant and to 2.67 *106 UFC/ g soil for the version with *B. officinalis* (Figure 6).

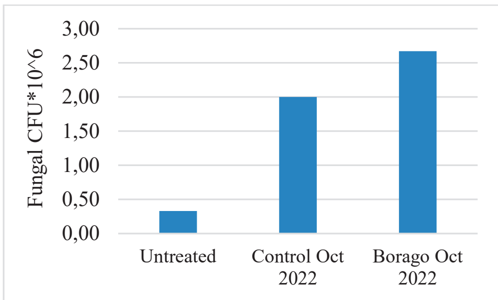

Figure 6. Fungal CFU evolution in soil samples

CONCLUSIONS

The results pointed out that the number of bacteria in the soil covered with flower strips increased significantly during the vegetation period for borage strips. In addition, borage proved to be a companion species that inhibit weeds growth successfully, including in high infestation of johnsongrass. After the first year, a significant decrease of soil pH was observed, bringing this parameter much closer to the optimal value for strawberry crop. Also, using borage as a companion plant, the amounts of soil carbon and nitrogen increased significantly, which led to an intense microbial activity.

The number of fungal CFU was very low compared to bacterial CFU, but the fact that *Borago* positively influenced the increase in the number of microorganisms in the soil is a strong point of this experiment.

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BIOCHEMISTRY OF JUJUBE FRUIT (*ZIZIPHUS JUJUBA* MILL.) FROM ANALYSES TO COMPOUNDS (REVIEW)

Rareș Cosmin PÂNDARU[1], Ana Cornelia BUTCARU[2], Florin STĂNICĂ[1, 2]

[1]Faculty of Horticulture, [2]Research Centre for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine, 59 Mărăşti Blvd, Dstrict 1, 011464, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

Jujube (Ziziphus jujuba Mill.) is one of the most appreciated species in China with a long hystory in the Eastern Asia. Consumers prefer to eat the jujube fruit fresh due to its sensory qualities, but also of its rich nutritional properties. Studies have shown that the most advantageous nutritional characteristics of jujube fruit were its content of soluble sugars, 2-3 times more than other fruits, vitamin C with 100 times more than other fruits, vitamin B, acid triterpenoid, proline, polysaccharides, flavonoids, iron, potassium, calcium, and zinc. Jujube fruits contain a large amount of soluble dry matter that can exceed 30% when the total carbohydrate content exceeds 27%. Fruit acidity can vary between 0.3-1.0%. However, in the post-harvest period, ethylene can have adverse effects on fruits, such as senescence, rapid loss of quality, nutrients, and increased chances of attack by pathogens. For a longer consumption period, these fruits should be kept in a controlled atmosphere. This sudy aims to present a review focused on the biochemistry of jujube highlighted through researches.

Key words: *nutritional value, vitamin C, post-harvest period.*

INTRODUCTION

Jujube (*Ziziphus jujuba* Mill.) is one of the world's oldest cultivated fruit trees and the most important species in the large cosmopolitan family Rhamnaceae in terms of its economic, ecological, and social importance.
The consumers prefer both fresh and dry fruits as food or for pharmaceutical products, given the high number of nutrients, and amino acids (Xue et al., 2009; Choi et al., 2011).
Due to its biochemical composition, jujube fruit is also used as food, food additives, and flavoring (Li et al., 2006).
Ziziphus jujuba fruit shape varies from round, oval to elliptical. The fruit size ranges from that of a cherry to that of a plum (Markovski et al., 2015).
The epidermis of the fruit is thin, lustrous, and reddish-brown to chocolate in color (Soliman et al., 2013). The hue of the fruit clearly indicates the fruit's maturity.
Fruits with more than 50% dark brown colour are the best suited to consumption, regarding taste and juiciness.
The organoleptic characteristics are ordinary at full maturity, when the fruit is fully pigmented, but the fruits are more suitable for drying (Chen et al., 2015).
Total soluble content and total acidity can be used to determine the maturity stage (Yao, 2013).
The main soluble sugars in the fruit are fructose, glucose, and sucrose, which improve the sweet taste of the fruit (Pereek, 2013). Lignin, cellulose, and hemicellulose were mentioned in many studies as the primary dietary fibers contained in the fruit, with an essential role in food digestion. (Gao et al., 2013; Chen et al. 2018).
The vitamin C, flavonoids, carotenoids, and saponin content, which has antioxidative, anti-cancer, and anti-diabetes properties (Choi et al., 2012) recommend the fruit.
Its contents of sugar, vitamin B, cyclic nucleotide, proline, triterpene acids, potassium, iron, and zinc, were studied by Hu (2011) and Liu & Whang (2009).
Sugar, vitamin C, and cyclic adenosine monophosphate (cAMP) concentration was approximately 2, 100, and 1000 times that of apples. Polysaccharides, flavonoids, alkaloids, polyphenols, and pigments were also abundant in the fruit (Liu & Whang, 2019).

Dried jujube had carbohidrates 630-763% compared to fresh fruits, 23-32%, and sour jujube, 74.8%. In the fresh jujube vitam C was between 200-800 mg/100 g in the fresh fruit and 12-29 mg/100 g in the dried one. Protein content varied between 2.9-6.3% in the dried fruits comparted to 1.2% in the fresh jujube. Ca (mg/100 g) had values between 14 (fresh), 20-63 (dried), and 270 (sour jujube). The phosphor content was of 23 mg/100 g in the fresh jujube, 55-75 mg/100 g in the dried, and 59 mg/100 g in the sour jujube. This biochemical profile highlighted the high medicinal fruit quality.

The species of the genus *Ziziphus* entail 43 terpenoids, 31 saponins, 165 alkaloids, 151 flavonoids, and 40 other miscellaneous compounds (Ahmad et al. 2017; Ji et al., 2017). Terpenoids are abundant in the plants of the genus *Ziziphus*. To far, about 43 triterpenes have been isolated from the fruits, flowers, leaves, and seeds of *Z. celata*, *Z. spina Christi*, *Z. mauritiana*, *Z. jujuba*, *Z. lotus*, and other plants, with numerous triterpenes exhibiting potential biological properties (Ros et al., 2000; Ahmad et al., 2017). Furthermore, saponins, which are sugar conjugates of triterpenes, were found throughout the genus *Ziziphus*. Most plant species relate these compounds with defense functions. Saponins have several functional (emulsification, solubilization, foaming, sweetness, bitterness) and biological properties that have the potential to be used in a variety of applications, including food, cosmetics, and pharmaceutical industries, as well as soil bioremediation (Ji et al., 2017). There were approximately 31 saponins found in the roots, leaves, fruits, and seeds of *Z. mauritiana*, *Z. joazeiro*, *Z. jujuba*, *Z. spina christi*, and other plants. (Bozicevic et al., 2017; Dubey et al., 2019).

The food value of jujube is high, mainly due to the high soluble solids content exceeding the value of 30%, being an natural source as swetener.

The fruit’s acidity varies between 0.3 and 1.0%. Depending on the cultivar, it has a very high content of ascorbic acid (vitamin C), with values of 330-880 mg/100 g fresh weight. The content of vitamin P exceeds 1000 mg/100 g fresh weight (Ciocârlan, 2000).

Like in other species, the cultivar deaply influence the biochemical profile of the fruit.

Due to its flavor, crispness, and juiciness, Dongzao is one of the most popular jujube cultivat worldwide. The jujube for export is harvested between August and October, with September being the best harvesting month (Morley-Brunker, 2010). Because of the limited shelf life of the Dongzao cultivar, the market supply is relatively short.

Several approaches have been tried to extend the shelf life of this cultivar. Zong et al. (2005), for example, investigated the influence of a controlled environment. Their findings revealed that the regulated environment preserves the integrity of the fruit membrane and resistance to browning and fermentation, hence extending the fruit's shelf life. However, this preservation mode is expensive, increasing the product's cost and making it less competitive.

Another technique is to harvest and store the fruit sooner, at the white ripening stage. Although this process is affordable, the influence on the nutritional quality of the fruit is uncertain. As a result, it is critical to understand the effect of development stages on the nutritional quality of jujube fruit in order to determine the optimal time at which the fruit can be harvested to preserve its nutritious value. The research presented the nutritional composition and quality of the jujube Dongzao depending on the maturity stages. The results revealed that the maturity stage had a substantial effect on the nutritious contents of the fruit. The majority of essential elements generated in the fruit grew until the full-red stage, and those that fell from the white maturity stage to the full-red stage exhibited just a minor decrease at the semi-red stage. All the carbohydrates increased significantly and gradually from white to full-red stage in the jujube fruit. Sucrose increased by 106% from white to full-red, whereas glucose, fructose, L-rhamnose, mannose, galactose, and maltose grew by 56%, 48%, 39%, 64%, 65%, and 160%, respectively. Overall, these results showed an accumulation of soluble sugars, mostly sucrose, mannose, and galactose, over the fruit maturity stages Accordingly, semi-red maturity seems to be the most appropriate stage for harvesting the jujube fruit to preserve its bioactive compounds (Zhang et al., 2020).

At the Faculty of Horticulture from Bucharest, 10 genotypes were studied: R1P2, R1P7, R1P10 (Hu Ping Zao*), R2P7, R3P2, R3P3 (crack resistant), R3P4 (Hu Ping Zao*), R3P6, R3P10 (Taigu), and Dong Zao. Two clones of Hu Ping Zao (R2P8 and R3P4) and one genotype R3P8 were analyzed in dehydrated form (*clones of different origins), verifying whether or not this preservation method influences the quality parameters of the fruit.

Fresh fruits were harvested from the beginning of September until October. After morphological measurements, fruits were stored at 2-3°C and 90-95% relative humidity. Part of them was dehydrated using an Excalibur dehydrator for 20 hours at 45°C. The genotypes recorded a fruit weight between 5.89 g (R3P1 selection) to 28.57 g (Cheng TuoZao). Most of the analyzed genotypes had a solid soluble content higher than 30% Brix. Fruit content in minerals varied between 0.16% and 3.38%, with an average of 1.78%. The ascorbic acid content varied between 110.0 mg/100 g fw and 1020.0 mg/100 g fw (R1P11 selection), averaging 306.1 mg/100 g fw. Fruit acidity, expressed as malic acid, varied from 0.16% to 0.82% with an average of 0.36% (Stănică, 2000; Stănică & Vasile, 2008; Dicianu et al., 2017). The polyphenol content changed during fruit ripening. Thus, the highest polyphenols content was found in the fruit at the beginning of its formation and decreased with its maturation, all this time protecting against pathogens and pests (Wang et al., 2016; Shi et al., 2018).

Rahman et al. (2018) noted that 51.99-71.75% of the jujube is edible, with the edible part containing 4.43-6.01% protein, 82.35-89.63% carbohydrates, 45.64-88.97 mg/100g ascorbic acid, 0.48-0.63% lipids, 132.16-196.58 mg/100 g phenolics and 101.17-132.04 mg/100 g flavonoids in its dry matter (Hendek Ertop & Atasoy, 2018; Višnjevec et al., 2019).

Li et al. (2007) and Sunil (2013) described the composition of various jujube cultivars (Jinsixiaozao, Yazao, Jianzao, Junzao, Sanbianhong). Total phenols, minerals, and vitamins were determined in their experiment for the fruits of these cultivars; significant variation was found for water (17.38-22.52%), carbohydrate (80.86-85.63%), proteins (4.75%-6.86%), lipids (0.37-1.02%), soluble fibre (0.57-2.79%), insoluble fibre (5.24-7.18%), reducing sugar (57.61-77.93%), and ash (2.26-3.01%) (Pereek, 2013). Glucose and fructose were identied as major soluble sugars in all five cultivars, while rhamnose, sorbitol and sucrose were also present in lower amounts.

TRADITIONAL MEDICINAL USES AND POTENTIAL HEALTH BENEFITS OF JUJUBE

Jujube *(Zisiphus jujuba)* has been used as a traditional Chinese medicinal plant for many years for its various and numerous health benefits, including anti-inflammatory (Yu et al., 2012), anti-cancer (Plastina et al., 2012), gastro-intestinal protective (Huang et al., 2008), anti-oxidant (Cheng et al., 2012), anti-insomnia and neuro-protective properties (Yoo et al., 2010).

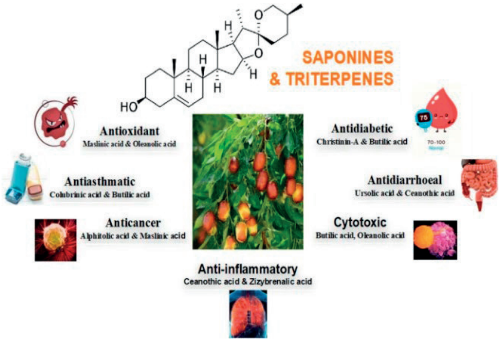

Figure 1. Main biological activities of triterpenes and saponins isolated from the genus *Ziziphus* (source: Sakna, 2022)

According to the modern medicine industry, jujube fruits and seeds are still utilized in Chinese and Korean traditional medicine and are said to relieve stress (Mill Goetz, 2009; Chen et al., 2017; Shahrajabian et al., 2020; Lu Y. et al., 2021). Among other health benefits, jujube lowers blood glucose and lipid levels and significantly lowers triglyceride, LDL, and cholesterol levels (Hemmati et al., 2015). Reche et al. (2019) observed 11 fatty acid compounds in four jujube cultivars, including, myristoleic acid, myristic acid, palmitic acid, cis-palmitoleic acid, trans-palmitoleic acid, stearic acid, oleic acid, 11-octadecenoic acid, linoleic acid, elaidic acid, and linolenic acid. Capric acid (C10:0), myristoleic acid (C14:1n5), lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1n9c), palmitic acid (C16:1n7), and linoleic acid (C18:2n6c) were found in four ripening phases of jujube fruit by

Song et al. (2019). Fatty acids are essential nutrients, some of which must be ingested through food to maintain health. The variety and content of fatty acids in jujube fruit could satisfy people's need for nourishment.

CONCLUSIONS

Jujube are sweet and nutritious, and have been used as dietary supplements since ancient times. For more than a thousand years, jujube has been used in China as a traditional herbal medicine for calming the mind. Modern scientific research has shown that the bioactive chemicals in jujube have anticancer, hepatoprotective, neuroprotective, antioxidant, anty-inflamatory and antiviral properties, as well as other health benefits such as enhancing immune function. Caution should be exercised when attempting to infer associations between the nutritional content and health functions of jujube. This is because the variety, ripening stage, storage and processing conditions of the fruit will affect its nutritional value and eventually lead to the increase or loss of some active functions.

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QUALITY ASSESMENT OF ORGANIC `PLAPINK` RASPBERRY FRUITS UNDER DIFFERENT STORAGE CONDITIONS

Andrei PETRE, Mihai FRÎNCU, Andreea BARBU (STAN), Andrei LESNIC, Liliana BĂDULESCU, Adrian ASĂNICĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihai.frincu@qlab.usamv.ro

Abstract

Raspberry (Rubus idaeus L.) is one of the most appreciated fruits in the world. According to FAO, the annual production in 2021 was about 886.000 t, with Europe which reached the largest input (approximately 68%). Due to the perishable nature of the fruits, there is a continuous need for improving the storage condition. The aim of the paper is to present the influence of storage conditions on organic raspberry fruits. Raspberries from `Plapink` cultivar, were harvested in 2021 and stored in three different conditions: 1) normal atmosphere (NA) with 1˚C and 85% relative humidity (RH), 2) controlled atmosphere (CA) conditions with 1˚C, 85% RH, 5% O_2 and 15% CO_2, and 3) CA conditions with 1˚C, 85% RH, 5% O_2, and 15% CO_2 for 3 days followed by NA for 6 days. Several quality indicators and physiological parameters were assed in order to verify the variations during the storage period. The total phenolic content values in NA decreased with about 6.28% compared to the initial moment, but no decreases were recorded during 3 days of storage in CA.

Key words*: raspberry, quality indicators, controlled atmosphere, bioactive compounds.*

INTRODUCTION

Worldwide, the total production of raspberries was more than 899 thousand of tons in 2020. The largest producers of raspberry fruits are in Russia, where more than 180 thousand tons are obtained annually, Mexico with more than 140 thousand tons and Poland with more than 123 thousand tons in 2020 (www.fao.org).

According to FAO, the total production of raspberries in Romania, in 2020 was 130 tons, on a cultivation area of 70 ha.

The special quality of the raspberry, as a result of the natural conditions specific to our country, has made this fruit more and more in demand on the international market, and as currently the highest production of fruit is obtained from the spontaneous flora, it is imperative the expansion of raspberry areas both in commercial plantations, exploited on modern bases, but also in family gardens. (Hoza, 2005).

Raspberries are very perishable fruits, being essential to be harvested at the right moment of maturating. (Haffner et al., 2002).

To minimize losses and prolong the postharvest longevity of organic raspberries, controlled atmosphere conditions are becoming more prevalent as a key postharvest technology. (Kruger et al., 2011).

Highly effective in extending the postharvest period of raspberries were the storage methods like cold, frozen and controlled atmosphere. (Forney et al., 2015).

Application of postharvest storage and processing techniques mentioned earlier, each operating under distinct parameters such as temperatures and processing times, could induce potential variations at biochemical, physical, biological levels (Bustos M.C. et al., 2018). As a result, these fluctuations may modify the content of bioactive chemical compounds in the fruit, ultimately impacting the nutraceutical properties valued by consumers (Michalska A. et al., 2015).

The aims of the paper are:

- monitoring and characterization of physico-chemical indicators and nutritional parameters of organic raspberry fruits during storage under normal and controlled atmosphere conditions;
- identification of optimal storage conditions in a controlled atmosphere for organic raspberry fruits.

MATERIALS AND METHODS

Samples

'Plapink' variety, harvested in 2021 was delivered to Posharvest Lab., being analysed before storage immediately after (initially moment).

The fruits were divided in three aliquots and stored in three different conditions:

1) normal atmosphere (NA) with 85% RH and 1˚C;
2) controlled atmosphere (CA) conditions with 15%CO_2, 5% O_2, 1°C, 85% RH
3) CA conditions with 15%CO_2, 5% O_2, 1°C, 85% RH for 3 days followed by NA for 6 days, similar with Haffner et al. 2002. Samples were analyzed in four moments in all storage conditions: initially, after 3, 6, and 9 days of storage.

Figure 1. Organic raspberries `PLAPINK` cultivar before storage

Quality indicators

Quality indicators followed were represented by: the average weight of 10 fruits, shape index, pH, total soluble solids (TSS), dry matter content, total titratable acidity (TTA), ascorbic acid content, total phenolic content (TPC) and antioxidant activity. The average weight of 10 fruits were measured using Radwag Partner PS 6000.R2 technical balance. Shape index were determined using a Parkside calliper, the results were reported using the formula h/D (h-height; D-transversal diameter). Using the index, the fruit form varies: shape index =1-round fruits; shape index ≤1-flattened fruits; shape index ≥1-elongated fruits. pH and TTA were determined with the pH electrode of automatic titrometer TitroLine easy. The procedure involves weighing around 5 g ± 10% of fresh homogenous sample, combining it with 25 mL of bi-distilled water, measuring the initial pH values, and subsequently titrating it with 0.1N NaOH until the pH value reaches 8.1, following AOAC Official Method 942.15. The TTA results were presented as grams of malic acid per 100 grams of fresh fruit, similar to the method described by Frîncu et al., 2023.

Total soluble solids (TSS) results were expressed using Kruss DR301-95 digital refractometer (Cătuneanu et al., 2017).

The dry matter content was determined by thermogravimetric method using a UN110 Memmert oven by drying 1 g ± 10% of the sample at 105°C, following the method similar with Stan et al. (2019), until constant weight was achieved.

Ascorbic acid was determined using HPLC method, similar with Stan et al., 2019.

Phenolic content and antioxidant activity

The quantification of total phenolic content (TPC) was performed using the Folin-Ciocâlteu method protocol. Initially, 1 g ± 10% of the fresh sample underwent trituration with 10 mL of 70% methanol, and the resulting mixture was incubated overnight in darkness at ambient temperature (approximately 22°C) within 15 mL centrifuge tubes. Subsequently, the extraction procedure extended to stir for 1 hour at 500 rpm followed by centrifugation at 7000 rpm at 4°C, for 5 minutes. The supernatant was collected in 50 mL tubes, and the residual material underwent two successive extractions until reaching a final extract volume of 30 mL. The initial step in the determination of TPC involved combining 0.5 mL of the extract with 2.5 mL of Folin–Ciocâlteu reagent, followed by a 2 minutes incubation at ambient temperature (approximately 22°C). The subsequent step entailed the addition of 2 mL of 7.5% sodium carbonate (Na_2CO_3), followed by incubation for 15 minutes at 50°C. The final step represents measuring the absorbance at a wavelength of 760 nm using a Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany). The results were noted in of mg GAE/100 g FW.

For the determination of antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, similar to the approach described by Bujor et al. (2016) with some adaptations. This involved combining 0.2 mL of the extract with 2 mL of a 0.2 mM solution of DPPH in methanol, followed by a 30 minutes incubation in darkness with homogenization. The absorbance of the resulting samples was measured at a wavelength of 515 nm, and the outcomes were expressed as mg Trolox/100 g FW, with methanol serving as the reference.

Statistical analysis

Standard deviation was the statistical analysis applied to all samples, representing the average from the same sample, of three replicates

RESULTS AND DISCUSSIONS

Quality indicators

The raspberry samples, both the fresh ones and the ones stored in the cold room and in the two controlled atmosphere conditions, were analyzed in order to determine their quality, following parameters such as: the average weight of 10 fruits, their caliber, pH, total soluble matter (% Brix), total dry matter (%), total titratable acidity, ascorbic acid content, total polyphenol content and antioxidant activity.

Thus, upon entering the cold room, the average weight of 10 fruits was 5.85 g, registering a 16% decrease until the last day of storage (Figure 2).

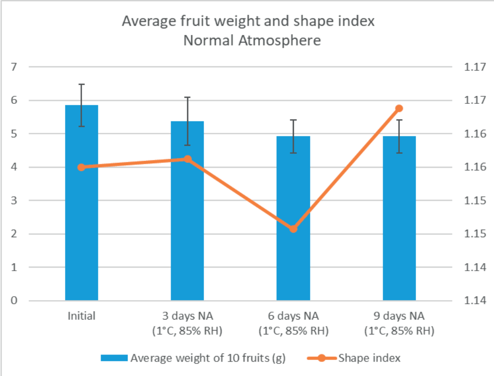

Figure 2. Average fruit weight and shape index values in Normal Atmosphere storage conditions

Before storage under controlled atmosphere conditions, the average weight of 10 organic raspberry fruits of the ‘Plapink’ variety was 5.29 g, registering a decrease of 23% by the last day of storage (Figure 3).

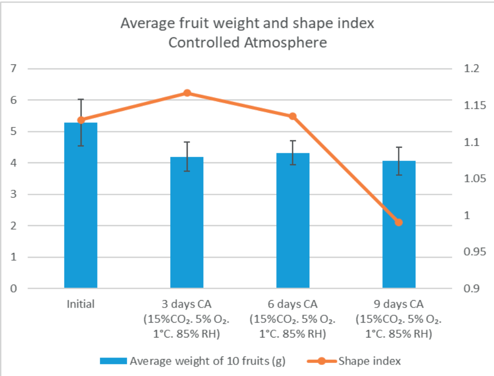

Figure 3. Average fruit weight and shape index values in Controlled Atmosphere storage conditions

Organic raspberry of the ‘Plapink’ variety was analyzed before storage in the controlled atmosphere room for 3 days, followed by storage in the cold room for 6 days. The average weight of 10 fruits was 5.29 g at the initial moment of analysis, registering a decrease of 18.3% after the 9 days of storage in the two experimental conditions (Figure 4).

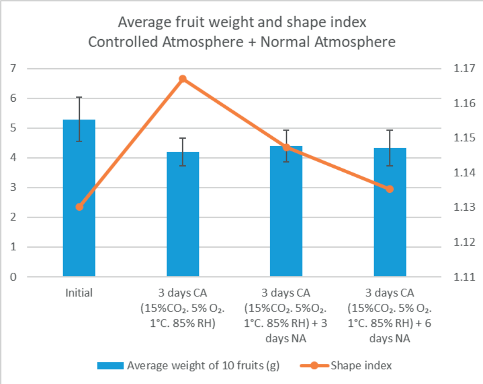

Figure 4. Average fruit weight and shape index values in Controlled Atmosphere followed by Normal Atmosphere storage conditions

Variations of pH and total titratable acidity were monitored during storage in the cold room yielding pH values of 2.82 initially moment and 2.83 after 9 days. The total titratable acidity at the initial moment of analysis was 2.21 mg malic acid/100 g fresh sample and 1.82 mg malic acid/100 g fresh sample after 9 days of storage in the cold room (Figure 5).

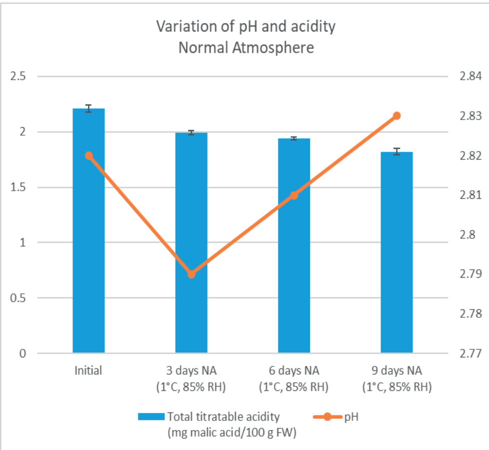

Figure 5. Variation of fruit pH and acidity in Normal Atmosphere storage conditions

Following the determinations made, after 9 days of storage in NA, the pH of the fruits registered a slight increase of 0.5% compared to the initial moment and the total titratable acidity registered a decrease of 17.64% compared to the moment initial.

Variations in pH and total titratable acidity were also monitored during controlled atmosphere storage with pH values of 2.73 at the initial moment and 2.72 after 9 days. The total titratable acidity at the initial moment was 2.00 mg malic acid/100 g fresh sample and 1.84 mg malic acid/100 g fresh sample after 9 days of storage in the cold room (Figure 6).

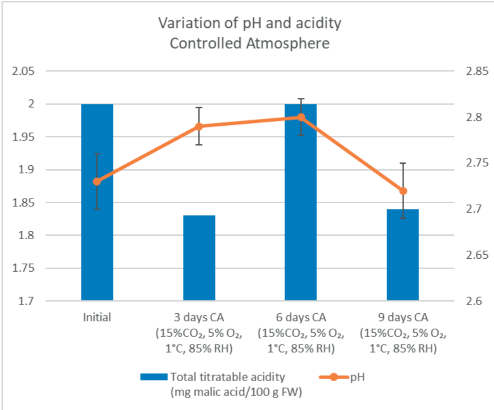

Figure 6. Variation of fruit pH and acidity in Controlled Atmosphere storage conditions

After 9 days of controlled room storage (15% CO_2, 5% O_2, 1°C, 85% RH), the pH of the fruits registered a slight decrease of 0.5% compared to the initial moment and the total titratable acidity decreased by 8% compared to the initial moment.

Changes in pH and total titratable acidity were also monitored during combined storage, controlled atmosphere for 3 days (15% CO_2, 5% O_2, 1°C, 85% RH), and normal cold room atmosphere for 6 days, obtaining pH values of 2.73 at the initial moment of analysis and 2.82 after 9 days. The total titratable acidity at the initial time of analysis was 2.00 mg malic acid/100 g fresh sample and 1.83 mg malic acid/100 g fresh sample after 9 days of storage in the cold room (Figure 7).

After 3 days of Normal Atmosphere storage followed by 9 days of Controlled Atmosphere storage (15% CO_2, 5% O_2, 1°C, 85% RH), the pH of the fruits registered a slight increase of 3.5% compared to the initial moment and the total titratable acidity decreased by 8.5% compared to the initial moment.

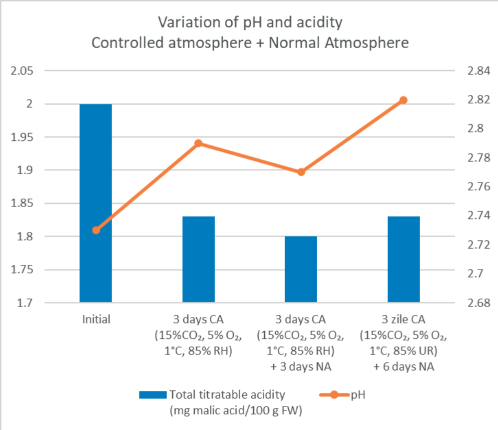

Figure 7. Variation of fruit pH and acidity in Controlled Atmosphere for 3 days followed by Normal Atmosphere storage conditions

Variations of total soluble matter (% Brix) and total dry matter (% DM) were monitored during storage in the cold room obtaining values of 11.40% for Brix and 10.87% SU when analyzing organic raspberry fruits at the initial moment (Figure 8).

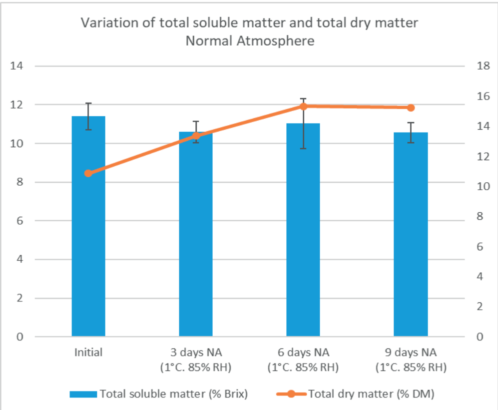

Figure 8. Variation of total soluble matter and total dry matter in Normal Atmosphere storage conditions

Following the determinations made, after 9 days of storage in a normal atmosphere in the cold room, the pH of the total soluble substance registered a decrease of 7.4% compared to the initial moment and the total dry matter registered an increase of 40.3% compared to the initial moment.

The variations of total soluble matter (% Brix) and total dry matter (% DM) were monitored during storage under controlled atmosphere conditions obtaining values of 10.66% for Brix and 12.74% DM when analyzing the fruits of organic raspberry at the initial moment (Figure 9).

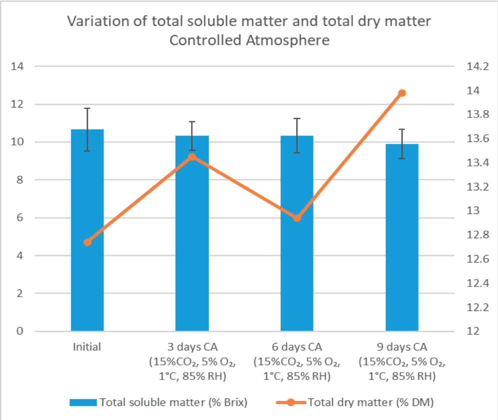

Figure 9. Variation of total soluble matter and total dry matter in Controlled Atmosphere storage conditions

After 9 days of controlled room storage (15% CO_2, 5% O_2, 1°C, 85% RH), the total soluble substance registered a decrease of 7.13% compared to the starting moment and the total dry matter registered an increase of 9.74% compared to the initial moment.

Variations in total soluble matter (% Brix) and total dry matter (% DM) were also monitored during combined storage, controlled atmosphere for 3 days and normal cold room atmosphere for 6 days, yielding values of 10, 66% for Brix and 12.74% DM when analyzing organic raspberry fruits at the initial moment and 9.68% for Brix and 13.94% DM after the 9 days of storage (Figure 10).

As a result of the experiment carried out, after 9 days of combined storage, controlled atmosphere for 3 days and normal atmosphere in the cold room for 6 days, it can be seen that the total soluble substance decreased by 9.2% compared to the initial moment, and the total dry matter registered an increase of 9.4% compared to the initial moment of the analysis.

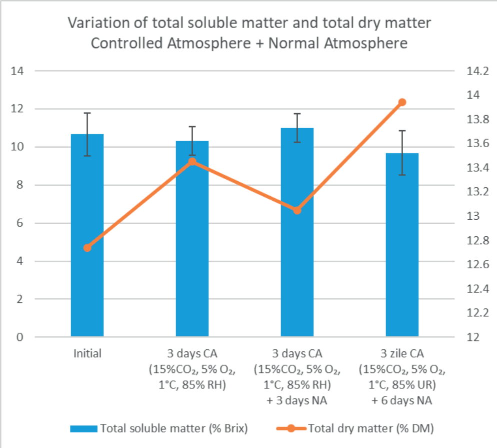

Figure 10. Variation of total soluble matter and total dry matter in Controlled Atmosphere followed by Normal Atmosphere storage conditions

Determining vitamin C (acid ascorbic content) in organic raspberries of the 'Plapink' variety at the time of harvesting and before being placed in the cold room (initial moment) recorded the value of 21.68 mg ascorbic acid/100g (Figure 11). After 3 days of storage in the cold room, vitamin C increased by 26.38% compared to the initial time, and by the end of storage in the normal atmosphere in the cold room, it increased by 46.4%. This increase in the content of vitamin C can be correlated with the concentration in the total dry matter of up to 40.3% compared to the initial moment which basically implies the dehydration of organic raspberry fruits due to storage in normal atmosphere in the cold room.

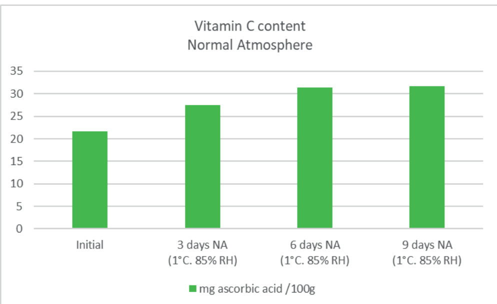

Figure 11. Vitamin C content - Normal Atmosphere storage conditions

The determination of ascorbic acid content in organic raspberries of the 'Plapink' variety at the time of harvest and before storage under controlled atmosphere conditions recorded the value of 20.76 mg ascorbic acid/100 g (Figure 12). After 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH), the vitamin C content increased by approximately 9.4% compared to the initial moment, but up to the end of the storage period presented a 5.5% decrease.

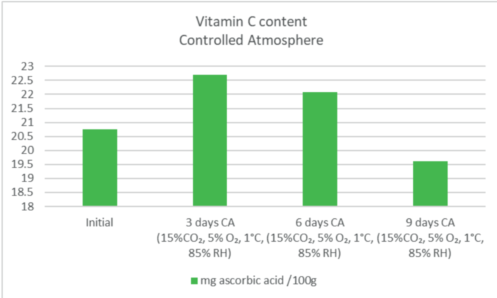

Figure 12. Vitamin C content - Controlled Atmosphere storage conditions

Vitamin C determination in organic raspberries of the 'Plapink' variety at the time of harvest and before combined storage (controlled atmosphere for 3 days and normal atmosphere in the cold room for 6 days), recorded the value of 20.76 mg of acid ascorbic/100 g (Figure 13).

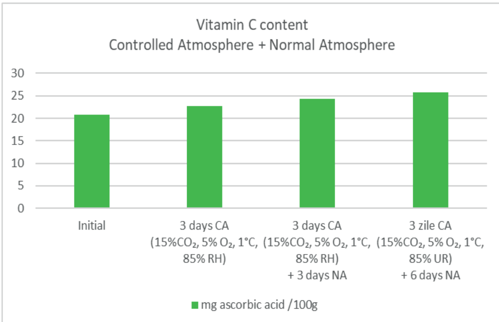

Figure 13. Vitamin C content in Controlled Atmosphere followed by Normal Atmosphere storage conditions

The 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH) were common with the second experimental variant, which is why the results obtained for vitamin C are identical. However, during the 6 days of normal atmosphere in the cold room, the vitamin C content increased by 24.13%, which also can be correlated with the total dry matter concentration during storage.

The determination of TPC (total phenol content) in organic raspberries of the 'Plapink' variety at the time of harvest and before being stored in the cold room (initial time) recorded the value of 183.33 mg GAE/100 g (Figure 14). After 3 days of storage in the cold room, TPC increased by 4.67% compared to the initial time, and by the end of the storage period in the normal atmosphere in the cold room, there was an increase of about 17%.

Regarding the antioxidant activity, for the initial moment, the value of 2373.46 mg Trolox equiv/100 g was recorded. After 3 days of storage in the cold room, the antioxidant activity recorded approximately similar values compared to the initial moment, and by the end of the storage period in the normal atmosphere in the cold room, it recorded an increase of approximately 12.57% compared to the initial moment, fact which can be determined, as in the case of total polyphenols, by the concentration of nutrients, implicitly the content of total polyphenols, due to the dehydration of the fruit during storage.

A similar behavior was also observed by Gunes et al. (2002) when they stored cranberries under normal atmospheric conditions for 2 months, noting that the antioxidant activity of the fruits increased by 50% compared to the time of harvesting. Moreover, they also observed that antioxidant activity values for fruits stored in CA (21% O_2 + 30% CO_2) remained constant, which may result from a bottleneck in the release of phytochemicals during storage in CA, which contributes to antioxidant activity.

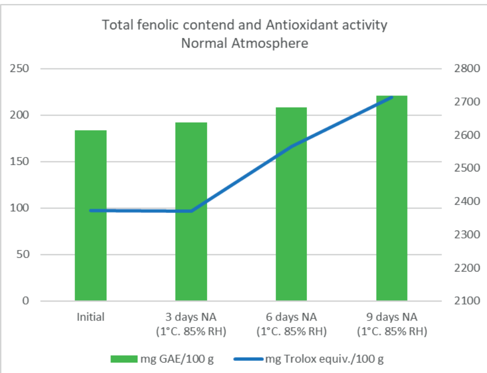

Figure 14. Total phenol content and Antioxidant activity of organic raspberries stored in Normal Atmosphere conditions

The determination of TPC in organic raspberries of the 'Plapink' variety at the time of harvest and before storage under controlled atmosphere conditions recorded the value of 229.94 mg GAE/100 g (Figure 15). After 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH), TPC decreased by approximately 6.3% compared to the initial moment. Still, by the end of the period of storage, the TPC registered a slight increase of approximately 7.5% compared to the initial moment.

Regarding the determination of the antioxidant activity in organic raspberries of the 'Plapink' variety at the time of harvest and before storage under controlled atmosphere conditions was recorded the value of 2865.32 mg Trolox equiv/100 g. After 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH), the antioxidant activity decreased by approximately 9.8% compared to the initial moment, but by the end of the period of storage the antioxidant activity registered a slight increase of 4.13% compared to the initial moment

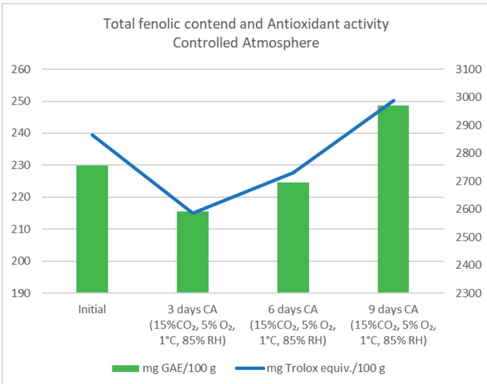

Figure 15. Total phenol content and Antioxidant activity of organic raspberries stored in Controlled Atmosphere conditions

The determination of TPC in organic raspberries of the 'Plapink' variety at the time of harvest and before combined storage, controlled atmosphere for 3 days and normal atmosphere in the cold room for 6 days, recorded the value of 229.94 mg GAE/100 g (Figure 16). The 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH), were common with the second experimental

variant, which is why the results obtained for polyphenols are identical. However, during the 6 days of normal atmosphere in the cold room, the TPC registered a slight decrease of about 6.28% compared to the initial moment.
The determination of the antioxidant activity in organic raspberries of the 'Plapink' variety at the time of harvest and before combined storage, controlled atmosphere for 3 days and normal atmosphere in the cold room for 6 days, recorded the value of 2865.32 mg Trolox equiv/100 g. The 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH), were common with the second experimental variant, which is why the results obtained for the antioxidant activity are identical. However, during the 6 days of normal atmosphere in the cold room, the antioxidant activity registered a decrease of approximately 9.85% compared to the initial moment.

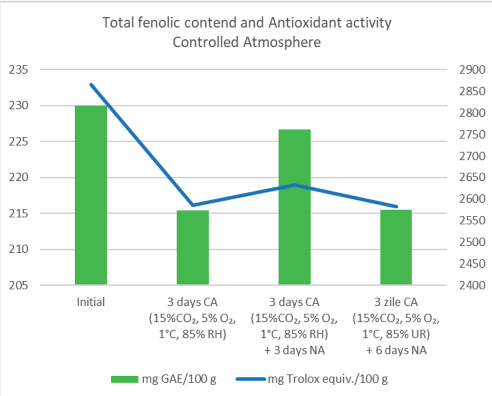

Figure16. Total phenol content and Antioxidant activity of organic raspberries stored in Controlled Atmosphere followed by Normal Atmosphere conditions

The determination of the antioxidant activity in organic raspberries of the 'Plapink' variety at the time of harvest and before combined storage, controlled atmosphere for 3 days and normal atmosphere in the cold room for 6 days, recorded the value of 2865.32 mg Trolox equiv/100 g (Figure 16). The 3 days of storage under controlled atmosphere conditions (15% CO_2, 5% O_2, 1°C, 85% RH), were common with the second experimental variant, which is why the results obtained for the antioxidant activity are identical. However, during the 6 days of normal atmosphere in the cold room, the antioxidant activity registered a decrease of approximately 9.85% compared to the initial moment.

CONCLUSIONS

In current experiment, all samples of organic raspberries present variation of quality indicators during the storage in all three conditions.
According to our results, it is recommended to store fruits in normal atmospheric conditions at a temperature of 1°C and a relative humidity of 85% for a maximum of 6 days. Longer storage will affect the nutritional and organoleptic qualities of the fruits.
Regarding storage in controlled atmosphere conditions with 5% O2 and 15% CO2, at a temperature of 1°C and a relative humidity of 85%, a maximum of 9 days can be achieved, the period in which the fruits maintain their optimal consumption qualities.

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EVALUATION OF BIOACTIVE COMPOUNDS WITH ANTIOXIDANT ACTIVITY OF *HELICHRYSUM ARENARIUM* (L.) MOENCH. INFLORESCENCES EXTRACTS

Gabriela POPA, Silvana DĂNĂILĂ-GUIDEA, Ecaterina DAMIAN, Gabriela MĂRGĂRIT, Diana GROPOȘILĂ-CONSTANTINESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464 Bucharest, Romania

Corresponding author email: popagabiro@yahoo.com

Abstract

The aim of this study was to evaluate the bioactive compounds with antioxidant activity in aqueous, 70% ethanolic and 80% methanolic extracts of Helichrysum arenarium inflorescences. Phytochemical screening was performed on the three extracts to detect the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, glycosides, phytosterols and tannins. The total flavonoid content was determined by aluminium chloride colorimetric assay at 420 nm. Total phenol content was determined with Folin-Ciocalteu reagent at 765 nm. Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and the total antioxidant capacity (TAC) assay. The results showed that among the three samples studied, the 70% ethanol extracts had the highest polyphenol and flavonoid values. Also, the antioxidant activity of the 70% ethanol extract was higher than that of the aqueous and 80% methanol extracts. The high content of phenols and flavonoids indicated that these compounds contribute to the antioxidant activity of Helichrysum arenarium. Phytochemical examination revealed the presence of alkaloids, flavonoids, phenols, glycosides, phytosterols and tannins in all extracts.

Key words: *Helichrysum arenarium, phenols, flavonoid, phytochemical screening, antioxidant activity.*

INTRODUCTION

Helichrysum arenarium (L.) Moench. (also known as “Sandy everlasting”, “Dwarf everlasting”, “Immortelle”, and “Siminoc” in popular Romanian folklore) belongs to the *Compositae* family and is an herbaceous perennial plant naturally distributed in Central, Eastern and South-Eastern Europe, North of Balkans, West Siberia, Central Asia, Mongolia, and China (Pljevljakušić et al., 2018). In Romania it grows in the spontaneous flora of the countryside, in sandy or calcareous places.
The plant is rich in bitter substances, tannins, flavonoids, saponins, apigenin, astragalin, colorants, glycosides, volatile oil, mineral salts. The pharmacological profile of *Helichrysum arenarium* has recently been improved by new research (Dănăilă-Guidea et al., 2022).
It revealed additional effects by identifying primary phytochemical ingredients that stimulate bile secretion production and circulation. (Les et al., 2017). The antioxidant, antidiabetic, and neuroprotective properties of *Helichrysum* species were investigated in order to obtain novel therapeutic products. Various studies regarding the composition of *Helichrysum arenarium* have shown that the presence of phenolic and flavonoid compounds provides antibacterial and antioxidant properties against pathogens (Czinner et al., 2000).
The inflorescences of *H. arenarium*, being rich in many bioactive compounds, have been used in European herbal medicine for its various beneficial properties for human health (Grădinaru et al., 2014). The *H. arenarium* essential oil demonstrated antimicrobial activity (Moghadam et al., 2014) against various microorganisms, including pathogenic strains. Other authors (Tepe et al., 2005) reported the antioxidant activity of the methanol extracts of various *Helichrysum* species.
H. arenarium plant was extensively investigated in different parts of the world. However, the plant has been less investigated in Romania in terms of bioactive compounds with antioxidant activity of inflorescence aqueous and alcoholic extracts. Therefore, the

aim of this study was to evaluate the bioactive compounds with antioxidant activity of inflorescences *Helichrysum arenarium* various extracts.

MATERIALS AND METHODS

Plant material. Dried inflorescences of *Helichrysum arenarium* (producer: Hypericum Impex Srl, Cluj-Napoca Romania) were purchased from Bucharest city commercial herb centres (Figure 1).

Figure 1. *H. arenarium* inflorescences material sources (producer: Hypericum Impex Srl, Cluj-Napoca, from Romania)

Samples preparation

Microwave-assisted extraction (MAE) method. The plant material consisting of dried flowers was weighed (1 g) and mixed with three different solvents (30 ml): 70% ethyl alcohol, 80% methanol and distilled water. Each sample (1 g/30 ml solvent) was placed in a microwave oven at 700 W, temperature = 85-90°C, extraction time 4 min (Pan et al., 2003). After microwave-assisted extraction, samples were cooled and filtered. The extracts were then analyzed for total phenol content, flavonoids and antioxidant activity.

Determination of total phenolic content (TPC). The concentration of phenolic compounds in the extracts was determined by the Folin-Ciocalteau method (Singleton, 1999). Samples were reacted with Folin- Ciocalteau reagent. After the formation of blue colored compounds, a Na_2CO_3 solution was added for alkalinization and the samples were then incubated for 2 h in the dark. After incubation, samples are measured spectrophotometrically (Eppendorf UV-Vis) at 750 nm against a similarly prepared control, but in which the sample extract is replaced by distilled water. For the determination of polyphenol concentration, a calibration curve was performed, starting from standard gallic acid solutions (50-500 mg/l). The concentration of phenols (mg GAE/l) in the samples was determined from the equation of the standard gallic acid curve ($y = 0.0009x + 0.0032$; $R = 0.9955$) obtained for different gallic acid concentrations (Figure 2).

The total phenolic content (TPC) in all samples was calculated using formula:

$$TPC = c \times V/M,$$

where:

TPC - Total phenol content (mg/g dry matter);
c = Concentration determined from the calibration curve (mg GAE/ml);
V = Extraction volume (ml);
M = Mass of plant material in the extract (g).

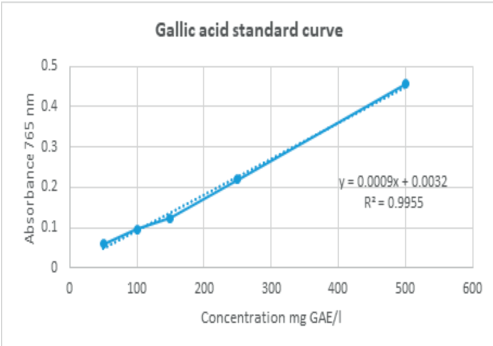

Figure 2. Gallic acid standard curve and the regression equation

Determination of total flavonoids content (TFC). The total content of flavonoids in the samples was determined by the colorimetric method with aluminum chloride (Chang et al., 2002). The reaction mixture consisted of: 1 ml sample/standard, 3 ml methanol, 200 µl AlCl3, 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 µg/ml) (Figure 3).

The total flavonoid components in the extracts in quercetin equivalents (QE) were calculated by the following formula:

TFC = C x V/M

where:

TFC = total flavonoid contents, milligram per gram of sample extract in QE;
C = the concentration of quercetin established from the calibration curve, mg/mL;
V = the volume of extract, milliliter;
M = the weight of sample extract (g).

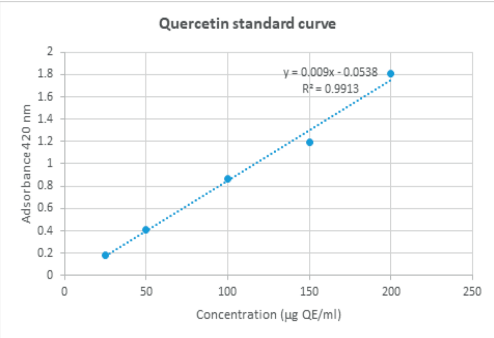

Figure 3. Quercetin standard curve and the regression equation

Phytochemical screening of extracts. Phytochemical screening was carried out on the three extracts to detect the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, phytosterols, glycosides and tannins as described in literatures (Kokate et al., 2006).

Between 0.5 and 1.0 ml of each extract was used for each phytochemical test. The results were expressed based on the intensity of the colour developed by the reaction and were noted with "+++" highly present, "++" moderately present, "+" low and "-" absent.

Antioxidant activity

To evaluate the antioxidant properties of the samples, two methods were applied to determine the antioxidant activity: the DPPH method and the phosphomolybdate method to determine the total antioxidant capacity.

DPPH (2,2-difenil-1-picrilhidrazil) method. The spectrophotometric method for assessing the total antioxidant capacity of the samples is based on the absorbance decrease of the DPPH radical in the presence of antioxidants.

The free radical scavenging activity of each sample was determined with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (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 (0.1mM). 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 (as blank) using a UV spectrophotometer (Eppendorf UV-VIS).

Different concentrations of ascorbic acid (10-200 µg/ml) were used as a standard agent.

The antioxidant capacity of the samples was expressed as inhibitory concentration, IC_{50} (µg/ml). The IC_{50} is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed. The lower IC_{50} value indicates the greater overall effectiveness of the antioxidant. The IC_{50} of the samples was measured by spectrophotometric method at the λ_{max} of DPPH, 517 nm.

The percent inhibition was calculated by the following equation:

$$\text{Inhibition}(\%) : [(A_{control} - A_{sample})/(A_{control})] \times 100$$

where: $A_{control}$ is the absorbance of the control reaction.

All tests were performed in triplicate. Concentration of samples resulting in 50% inhibition on DPPH (IC_{50} value) were calculated.

Total antioxidant capacity (TAC). The total antioxidant capacity of the samples was evaluated by phosphomolybdate method (Prieto et al., 1999) using ascorbic acid as a standard. 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.

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.

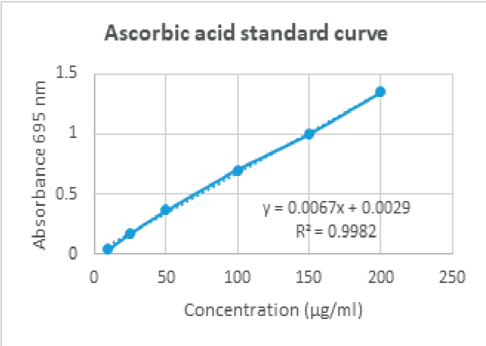

Figure 4. Ascorbic acid standard curve and the regression equation

RESULTS AND DISCUSSIONS

Microwave-assisted extraction (MAE). Dried flowers of *H. arenarium* were mixed with three different solvents: ethyl alcohol 70%, methanol 80% and distilled water. For extracting constituents from plant material, we used Microwave-assisted extraction (MAE) method.

In recent years, the use of the microwave oven for extracting constituents from plant material has shown tremendous research interest and potential.

Conventional techniques for extracting active constituents are time and solvent consuming, thermally unreliable, and the analysis of the many constituents in plant material is limited by the extraction step (Mandal et al., 2007). Traditional solvent extraction techniques for plant materials rely mainly on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of the desired compounds and improve mass transfer. Usually, the traditional technique requires a longer extraction time, which leads to a severe risk of thermal degradation for most phytoconstituents. The fact that a single plant can contain up to several thousand secondary metabolites makes the need to develop fast and high-performance extraction methods an absolute necessity (Nyiredy, 2004).

The use and development of new techniques with shorter extraction times, reduced solvent consumption, increased concern for pollution prevention and special attention to thermolabile constituents.

New extraction methods including microwave-assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized solvent extraction (PSE) have attracted significant attention over the last decade.

Total phenolic and flavonoids content. The results for total phenolic and flavonoids content of *H. arenarium* extracts are shown in Figure 5. The total phenolic content of the 70% ethanol (19.26 ± 0.193 mg GAE/g dm) and 80% methanolic (11.48 ± 0.095 mg GAE/g dm) extracts was higher than the aqueous extract sample. Similarly, the content of flavonoids for both alcoholic extracts was greater (59.58 ± 0.125 mg QE/g dm in 70% ethanol and 50.48 ± 0.168 mg QE/g dm in 80% methanolic extracts) than that of aqueous extract (30.99 ± 0.199 mg QE/g dm) (Figure 5; Table 2).

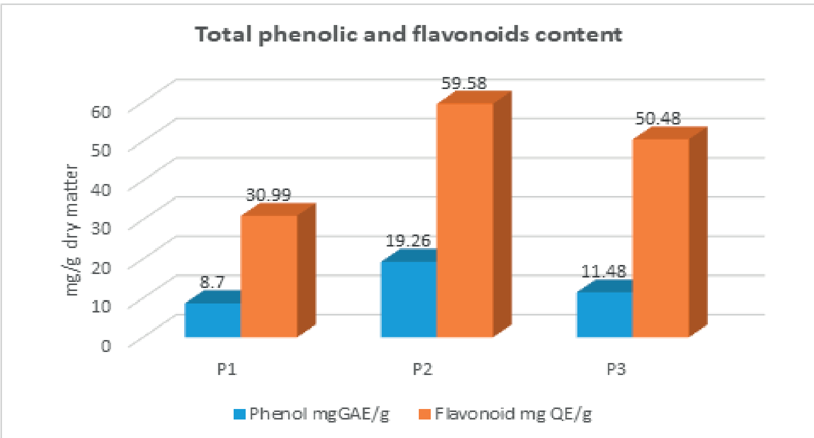

Figure 5. Total phenolics and flavonoids content in *H. arenarium* extracts
P1 - aqueous extract; P2 - 70% ethanol extract; 80% methanol extract

Czinner et al. (1999) suggested that the choleretic and hepatoprotective activities of *Helichrysum arenarium* inflorescence could be attributed to the antioxidant properties of its phenolic and flavonoid compounds. Various studies have revealed the presence of numerous bioactive compounds in *Helichrysum arenarium* inflorescences that have medicinal properties used in European phytotherapy (Grădinaru et al., 2014).

Phytochemical screening

Phytochemicals are currently enjoying increased attention due to exciting new findings on their biological activities (Cho et al., 2003). Alkaloids, flavonoids, phenols, glycosides, phytosterols and tannins detected in these extracts could implicate these phytochemicals as important bioactive agents in therapeutic action (Aliyu et al., 2013). The high phenolics content found in alcoholic extracts indicates high antioxidant potentials because the phenolics constituents can react with active oxygen radicals such as hydroxyl radical (Hussain et al., 1987), superoxide anion radical (Afanasev et al., 1989) and lipid peroxyl radical. The alcoholic extracts were found to have phenolic contents of 19.26 mg GAE/g dm for ethanol and 11.48 mg GAE/g dm and for methanol (Figure 5).

Phytochemical examination revealed the presence of alkaloids, flavonoids, phenols, glycosides, phytosterols and tannins in all extracts (Table 1, Figure 6).

Table 1. Phytochemical profile of *H. arenarium* extracts

Phytoconstituent	Reagent/test	Color change	Presence		
			Aqueous extract	Ethanolic extract	Methanolic extract
Alkaloids	Wagner	Reddish-brown	++	+++	+
Flavonoids	Alkaline test	Intense yellow	+++	+++	++
Phenols	Folin-Ciocalteu	Dark blue	++	+++	+++
Glycosides	Keller – Killani	Brown/green ring	++	++	++
Phytosterols	Salkowsky	Reddish-brown/Yellow	+++	++	+
Tannins	Ferric chloride test	Brown	++	+++	+++

Note: +++: highly present, ++: moderately present, +: low, -: absent.

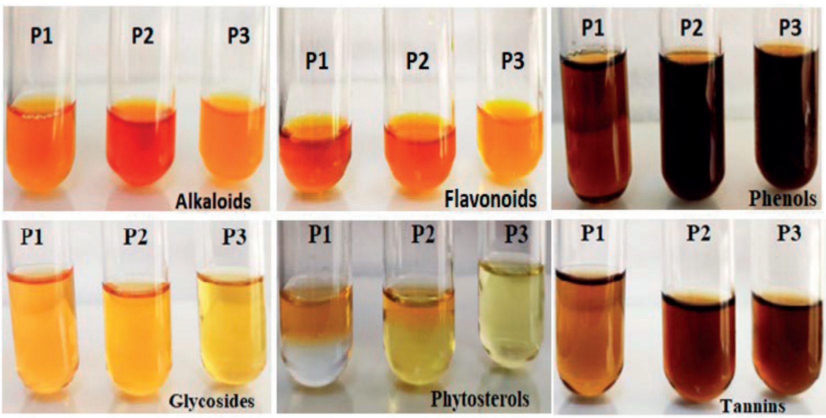

Figure 6. Presence of phytochemical compounds in *H. arenarium* extracts
P1 - aqueous extract; P2 - 70% ethanol extract; 80% methanol extract

Antioxidant activity

The antioxidant activity of *H. arenarium* extracts was determined via two methods (DPPH method and Total antioxidant capacity (TAC) by phosphomolybdate method). Assay based upon the use of DPPH radicals is among the most popular spectrophotometric methods for determination of the antioxidant capacity of plant extracts because the radical compounds can directly react with antioxidants. Additionally, DPPH scavenging method has been used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedures (Gonçalves et al., 2005).

In the present study, the $IC_{50\%}$ value of ascorbic acid, a well-known potent antioxidant, was 71.36±0.614 µg /ml.

The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acid pH.

The anti-radical activity of the samples with 70% ethanol displayed higher values (DPPH $IC_{50\%}$ = 109.5±0.341 µg /ml and TAC 202±11.78 µg /ml) than the samples with 80% methanol (DPPH $IC_{50\%}$ = 125.7±0.344 µg /ml and TAC 39.13±9.011 µg /ml) and distillated water (DPPH $IC_{50\%}$ = 151.2±0.263 µg/ml and TAC 9.41±0.129 µg /ml) by both methods. These results were presented in Figure 7 and Table 2.

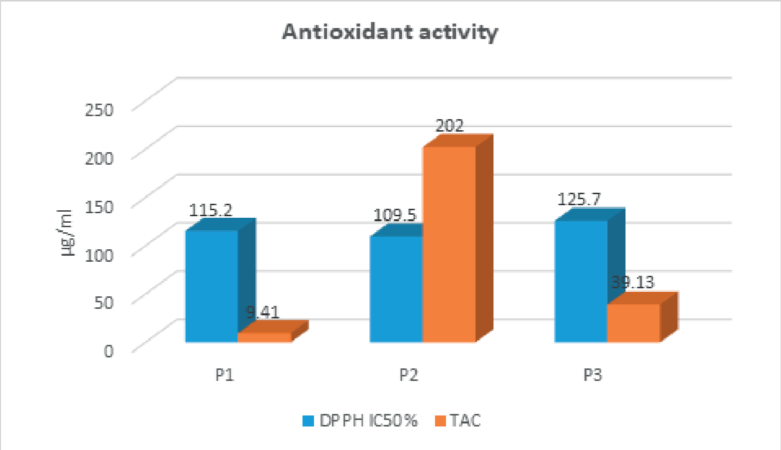

Figure 7. Antioxidant activity of *H. arenarium* extracts

The results for ethanol extracts and their antioxidant activity were in accordance with the total phenolic content. (TPC) and the concentration of flavonoids (TFC) (Table 2).

Table 2. Quantitative analyses of *H. arenarium* extracts

Sample	TPC mg GAE/g dm	TFC mg QE/g	DPPH IC50% µg /ml	TAC µg /ml
P1	8.70±0.087	30.99±0.199	151.2±0.263	9.41±0.129
P2	**19.26±0.193**	**59.58±0.125**	**109.5±0.341**	**202±11.78**
P3	11.48±0.095	50.48±0.168	125.7±0.344	39.13±9.011

Data are mean ± SEM for triplicate measurements
P1 - aqueous; P2 - 70% ethanol; 80% methanol

Various extracts (methanolic, ethanolic and 70% v/v ethanolic extracts) of Romanian sandy everlasting (harvested from Botoșani county), containing high amounts of polyphenols, have been tested for antioxidant properties (Babotă et al., 2018).

CONCLUSIONS

The 70% ethanol extract of *H. arenarium* showed the highest total flavonoid content and total phenolic content compared to other samples investigated. Moreover, ethanolic extract has highest DPPH free radical scavenging activity and TAC which could be related to its higher phenolic content. Phytochemical examination revealed the presence of alkaloids, flavonoids, phenols, glycosides, phytosterols and tannins in all extracts. The presence of various bioactive compounds in *Helichrysum arenarium* inflorescences may have beneficial properties for human health.

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PRELIMINARY RESULTS REGARDING THE SELECTION OF NEW BLUEBERRY GENOTYPES (*VACCINUM CORYMBOSUM* L.)

Dan Nicolae POPESCU, Cosmin Alexandru MIHAI, Ana Cornelia BUTCARU, Mihaela IORDĂCHESCU, Adrian ASĂNICĂ

University of Agronomic Sciences and Veterinary Medicine, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: cosmin.mihai@horticultura-bucuresti.ro

Abstract

Highbush blueberry (Vaccinium corymbosum L.) is a significant specie in terms of economical, nutritional, and medicinal point of view. Beside these attributes it is well known for its high anthocyanin content and antioxidant activity. Therefore, obtaining new valuable blueberry genotypes resilient to climatic changing conditions is a priority for breeders. The genotypes studied were obtained by a classical breeding method, respectively by free pollination, the seeds being prior cold stored and then sown in seedlings trays with acidic peat. Germination lasted even two years for some genotypes. The study presents the first phenotypic results for the obtained genotypes, highlighting differences and similarities regarding the foliar system and health status. Thirteen local (including 'Safir', 'Compact', 'Simultan') and international (Duke, Pink Lemonade, Berkeley, etc.) blueberry cultivars were used as parents. The results enclose twenty hybrids obtained from free pollination.

***Key words:** highbush blueberry, high chill cultivars, genetic variability, blueberry breeding.*

INTRODUCTION

Blueberries (*Vaccinium* spp.) are one of the most economically significant and nutritionally valuable fruit crops worldwide (Edger et al., 2022). The demand for these antioxidant-rich berries has fuelled research endeavours to enhance cultivation practices, optimize yield, and improve fruit quality (Mladin et al., 2008; Patrick & Li, 2017; Hera et al.,2021; Edger et al., 2022; Babiker et al., 2023).

Core to these efforts is the field of phenotyping, a comprehensive approach that integrates genetic, physiological and environmental factors to characterize the measurable traits of blueberry plants throughout their development (Verde et al., 2013; Cândea-Crăciun et al., 2018; Manzanero et al., 2023). In recent years, the application of advanced phenotyping technologies has emerged as a transformative force in understanding the intricate genetic and physiological mechanisms governing blueberry growth and productivity. Phenotyping, broadly defined as assessing observable traits, offers a holistic perspective beyond conventional genetic studies. It involves the measurement and analysis of morphological, biochemical, and molecular characteristics, providing valuable insights into the complex interplay between genotype and environment (Lobos & Hancock, 2015; Asănică et al., 2017; Franeti et al., 2020).

Current article explores the recent strides in blueberry phenotyping, shedding light on the innovative methodologies and technologies driving progress in the field. By delving into the intricacies of blueberry phenotypic characterization, researchers aim to disclose the mysteries surrounding the dynamic responses of these plants to environmental stimuli, stressors, and genetic variations. Through the lens of phenotyping, we aim to elucidate the intricate interplay between genotype and phenotype in blueberries, offering insights that can guide breeding programs toward developing better cultivars with enhanced nutritional profiles, improved tolerance to different stressors, and increased adaptability to diverse growing environments. Integrating advanced phenotyping techniques marks a paradigm shift in blueberry research, unlocking new avenues for sustainable and resilient berry production in the face of a changing climate and evolving market demands.

MATERIALS AND METHODS

The biological material involved in the present work was represented by foreign cultivars (‘Northland’, ‘Bluetta’, ‘Berkely’, ‘Coville’, ‘Draper’, ‘Duke’, ‘Nelson’, ‘Patriot’, ‘Spartan’, ‘Pink Lemonade’) and Romanian ones (‘Simultan’, ‘Compact’, ‘Lax’ ‘Safir’) from the blueberry collection of the Faculty of Horticulture Bucharest. The blueberry collection is set up in containers where the soil-specific properties could be better satisfied. From the above blueberry cultivars fruits, as a result of free pollination, seeds were extracted, passed through the process of stratification in cold rooms, and later sown and grown into small pots. Some seeds germinated after two years. Two and three years after germination, one and three mature hybrids of each cultivar were obtained. In the phenotyping process, five leaves were collected for both cultivars and hybrids that were morphologically analyzed with the WinFolia system. WinFolia system included an Epson scanner and software for image analyses that accurately could measure the principal biometrical leaves parameters. It has been designed explicitly for analyzing leaves in terms of leaf morphology and, including color codes, to deliver the rate of the disease foliar percentage.

Microsoft Excel 2016 and IBM SPSS v. 28.0.1.1 software were used for the statistical analyses of the data with a significance level of p = 0.05 were used.

RESULTS AND DISCUSSIONS

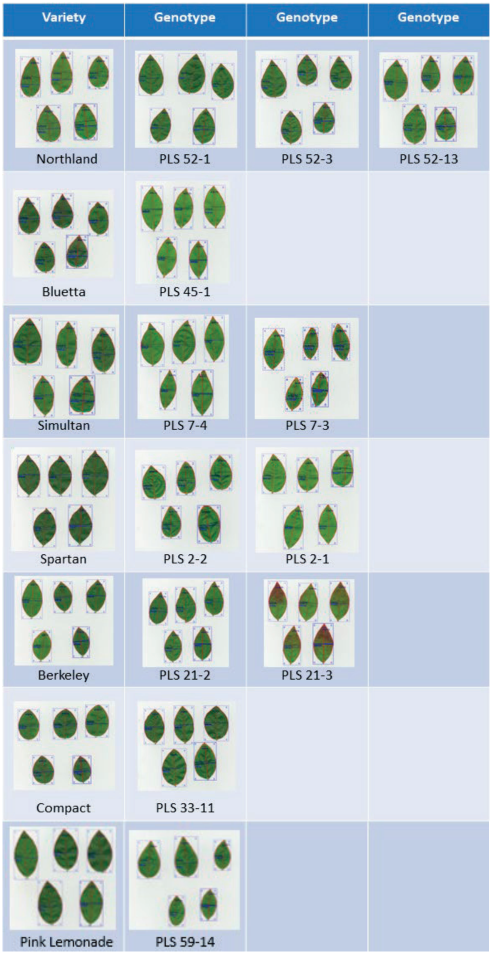
The first data included the visual analysis of the hybrids compared to the genitors. After scanning with the WinFolia program, images were obtained with the five leaves for each cultivar and hybrid. In Table 1, the mother genitor and the corresponding hybrids were presented. They are valuable for future applications for plant/cultivar/hybrid recognition.

In the second phase, morphological parameters were analyzed for hybrids and cultivars. WinFolia software delivers results on leaf area, perimeter, vertical length, width, ratio (W/L), form coefficient, blade length, lobe angles, and petiole length and area (Tables 2 and 3).

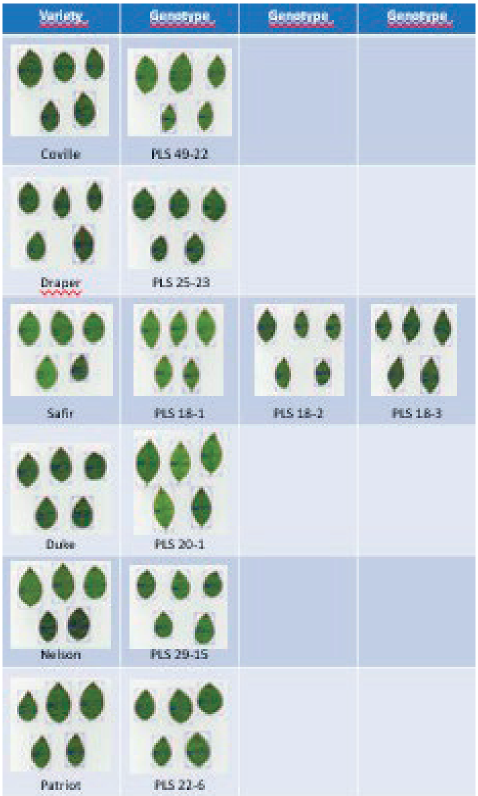
When analysing the leaf area, hybrids proved to have a large variability, the values being between 5.488 (PLS 7-3) and 23.218 cm^2 (PLS 52-1), the same characteristics being observed in the other parameters.

For the petiole length, there were no significant differences between variants. For the petiole area, PLS 2-1 had the highest value (0.016 cm^2), followed by PLS 18-3, PLS 25-23, PLS 22-6, PLS 52-13, PLS 7-4, PLS 2-2, PLS 59-14, PLS 45-1, PLS 20-1, PLS 18-1, PLS 33-11 (no significant differences between them). The group of PLS 7-3, PLS 18-2, PLS 29-15, PLS 52-3, PLS 49-22, PLS 21-2, PLS 52-1, PLS 21-3 had lower values.

Table 1. Cultivars and the corresponding hybrids analyzed with the WinFolia program



Variety	Genotype	Genotype	Genotype
Northland	PLS 52-1	PLS 52-3	PLS 52-13
Bluetta	PLS 45-1		
Simultan	PLS 7-4	PLS 7-3	
Spartan	PLS 2-2	PLS 2-1	
Berkeley	PLS 21-2	PLS 21-3	
Compact	PLS 33-11		
Pink Lemonade	PLS 59-14		



For the healthy status of the plants, Winfolia software was used with image analysis based on color. At the hybrids, all the variants presented similar values (Table 2), except PLS 21-3, which had a lower value (71.37%). In general, hybrids had very good, healthy foliage. Based on the morphological parameters, hybrids were analyzed in clusters (Figure 1). According to the obtained dendrogram, five common groups were obtained. First group included PLS 52-3, PLS 18-1, PLS 29-15, PLS 2-2, second group PLS 25-23, PLS 22-6, PLS 33-11, PLS 52-1, third group PLS 21-3, PLS 18-3, PLS 45-1, PLS 7-4, fourth group PLS 52-13, PLS 59-14, PLS 21-2, and the fifth group PLS 2-1, PLS 18-2, PLS 49-22, PLS 7-3. The first and second groups shared characters through the hybrid PLS 29-15 with PLS 33-11. Groups three and four shared characters through the hybrid PLS 59-14 with PLS 18-2.

Based on the morphological parameters, cultivars were analyzed in clusters (Figure 2). According to the obtained dendrogram, four common groups were obtained. The first group included Coville PLS 19, Patriot PLS 22, and Nelson PLS 29; the second group Simultan PLS 7, Draper PLS 25, and Berkeley PLS 21; the third group Lax PLS 13, and Safir PLS 18; the fourth group Bluetta PLS 45, Northland PLS 52, and Duke PLS 20.

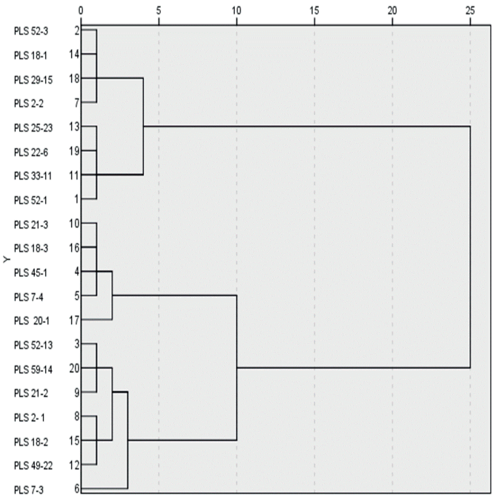


Figure 1. Hybrids grouped in clusters based on the morphological parameters

When the cultivars were analyzed (Table 3), the same variability was observed for the leaf area, where values ranged between 96.169 (Bluetta PLS 45) and 194.795 cm^2 (Pink Lemonade PLS 9) (results expressed for the five leaves). There was a slight variability for petiole length; the values ranged from 0.232 (Bluetta PLS 45) to 0.520 cm (Pink Lemonade PLS 9).

The petiole area recorded the highest value for Pink Lemonades PLS 9 (0.091 cm^2). The lowest value for the Bluetta PLS 45 cultivar (0.026 cm2), followed upwards by Draper PLS 25, Northland PLS 52, Compact PLS 33, Berkeley PLS 21, Duke PLS 20, Simultan PLS 7, Safir PLS 18, Patriot PLS 22, Lax PLS 13, Coville PLS 19, Nelson PLS 29, and Spartan PLS 2.

Table 2. Biometric data variability for the studied hybrids

Hybrid code	Leaf area	Perimeter	Vertical length	Width	Healthy status (%)	Petiole Length	Petiole Area
PLS 7-3	5.488 ^{h}	96.147 ^{h}	41.182 ^{i}	19.812 ^{f}	713.740 ^{b}	.0586 ^{a}	.0050 ^{b}
PLS 2-2	10.007 ^{g}	123.052 ^{g}	47.989 ^{gh}	28.990 ^{e}	955.300 ^{a}	.0594 ^{a}	.0055 ^{b}
PLS 59-14	10.375 ^{g}	127.278 ^{g}	51.274 ^{gh}	29.024 ^{e}	969.600 ^{a}	.0631 ^{a}	.0057 ^{b}
PLS 52-13	10.715 ^{g}	129.527 ^{fg}	52.392 ^{fgh}	29.464 ^{e}	971.180 ^{a}	.0647 ^{a}	.0061 ^{b}
PLS 21-2	11.286 ^{fg}	133.093 ^{efg}	53.476 ^{fgh}	29.837 ^{e}	971.660 ^{a}	.0675 ^{a}	.0064 ^{b}
PLS 18-2	11.313 ^{fg}	135.459 ^{efg}	54.322 ^{fgh}	30.108 ^{de}	980.140 ^{a}	.0688 ^{a}	.0066 ^{b}
PLS 29-15	11.971 ^{efg}	137.358 ^{defg}	55.135 ^{efg}	30.243 ^{de}	980.900 ^{a}	.0710 ^{a}	.0072 ^{b}
PLS 21-3	12.270 ^{defg}	137.591 ^{defg}	55.812 ^{efg}	30.582 ^{de}	981.080 ^{a}	.0774 ^{a}	.0073 ^{b}
PLS 45-1	12.850 ^{defg}	141.390 ^{cdefg}	57.268 ^{defg}	32.343 ^{cde}	982.840 ^{a}	.0800 ^{a}	.0074 ^{ab}
PLS 18-1	12.912 ^{defg}	146.856 ^{cdef}	59.267 ^{def}	32.546 ^{cde}	983.280 ^{a}	.0808 ^{a}	.0079 ^{ab}
PLS 52-3	13.245 ^{defg}	147.865 ^{cdef}	61.773 ^{cde}	33.122 ^{cde}	983.620 ^{a}	.0867 ^{a}	.0086 ^{ab}
PLS 2- 1	13.592 ^{defg}	148.485 ^{cdef}	61.807 ^{cde}	33.765 ^{cde}	984.060 ^{a}	.0887 ^{a}	.0090 ^{ab}
PLS 18-3	13.818 ^{defg}	149.144 ^{cdef}	63.161 ^{cd}	34.273 ^{cde}	984.540 ^{a}	.0921 ^{a}	.0094 ^{ab}
PLS 25-23	15.067 ^{cdef}	152.090 ^{cde}	63.500 ^{cd}	34.612 ^{cde}	984.920 ^{a}	.0926 ^{a}	.0098 ^{ab}
PLS 49-22	15.184 ^{cdef}	156.122 ^{bcd}	64.448 ^{cd}	35.594 ^{cd}	986.240 ^{a}	.0940 ^{a}	.0103 ^{ab}
PLS 33-11	15.721 ^{bcde}	157.712 ^{bc}	64.482 ^{cd}	36.881 ^{c}	987.060 ^{a}	.1026 ^{a}	.0111 ^{ab}
PLS 7-4	16.110 ^{bcd}	159.650 ^{bc}	68.275 ^{b}	37.287 ^{bc}	989.940 ^{a}	.1108 ^{a}	.0112 ^{ab}
PLS 22-6	18.207 ^{bc}	171.630 ^{ab}	72.678 ^{ab}	37.694 ^{bc}	991.000 ^{a}	.1142 ^{a}	.0123 ^{ab}
PLS 20-1	18.961 ^{b}	182.756 ^{a}	74.710 ^{ab}	42.096 ^{ab}	1.000.000 ^{a}	.1201 ^{a}	.0132 ^{ab}
PLS 52-1	23.218 ^{a}	185.819 ^{a}	79.011 ^{a}	45.821 ^{a}	1.000.000 ^{a}	.1418 ^{a}	.0161 ^{a}

*a-h are corresponding to the post-hoc Duncan test analysis

Table 3. Biometric data variability for the cultivar used as parents in the study

Cultivar code	Leaf area	Perimeter	Vert Length	Healthy	Petiole Length	Petiole Area
Bluetta PLS 45	96.169 ^{f}	118.749 ^{e}	48.328 ^{f}	407.560 ^{b}	.2319 ^{c}	.0259 ^{c}
Draper PLS 25	111.356 ^{ef}	123.246 ^{e}	48.768 ^{f}	523.700 ^{b}	.2583 ^{bc}	.0304 ^{c}
Northland PLS 52	111.789 ^{ef}	126.968 ^{e}	53.408 ^{ef}	745.260 ^{a}	.3077 ^{abc}	.0316 ^{c}
Compact PLS 33	118.053 ^{def}	128.674 ^{de}	53.577 ^{ef}	823.740 ^{a}	.3088 ^{abc}	.0374 ^{c}
Berkeley PLS 21	122.679 ^{def}	130.574 ^{de}	54.932 ^{def}	856.260 ^{a}	.3527 ^{abc}	.0454 ^{bc}
Duke PLS 20	126.651 ^{cdef}	132.434 ^{de}	57.641 ^{cde}	867.360 ^{a}	.3579 ^{abc}	.0484 ^{bc}
Simultan PLS 7	139.347 ^{bcde}	146.197 ^{cd}	58.623 ^{cde}	891.340 ^{a}	.3766 ^{abc}	.0501 ^{bc}
Safir PLS 18	151.869 ^{bcd}	146.236 ^{cd}	61.536 ^{bcd}	905.520 ^{a}	.4016 ^{abc}	.0509 ^{bc}
Patriot PLS 22	159.347 ^{abc}	153.176 ^{bc}	63.432 ^{bc}	942.640 ^{a}	.4186 ^{abc}	.0559 ^{abc}
Lax PLS 13	162.810 ^{ab}	154.533 ^{abc}	63.669 ^{bc}	963.360 ^{a}	.4477 ^{abc}	.0583 ^{abc}
Coville PLS 19	168.732 ^{ab}	158.255 ^{abc}	63.906 ^{bc}	978.180 ^{a}	.4537 ^{abc}	.0605 ^{abc}
Nelson PLS 29	193.175 ^{a}	161.860 ^{abc}	68.682 ^{ab}	982.940 ^{a}	.4667 ^{ab}	.0747 ^{ab}
Spartan PLS 2	193.446 ^{a}	168.218 ^{ab}	73.355 ^{a}	984.740 ^{a}	.4799 ^{ab}	.0753 ^{ab}
Pink Lemonade PLS 9	194.795 ^{a}	172.250 ^{a}	73.389 ^{a}	989.060 ^{a}	.5203 ^{a}	.0911 ^{a}

*a-f are corresponding to the post-hoc Duncan test analysis

The leaf perimeter recorded the lowest value of 118.749 cm for the Bluetta PLS 45 cultivar, increasing significantly to 172.25 for the Pink Lemonade PLS 9. Vertical length has no significant values in terms of variability (data expressed for five leaves).
The WinFolia program used to analyze leaf health highlighted the Bluetta PLS 45 cultivar with the lowest value, having specific signs of disease, and the Pink Lemonade PLS 9 cultivar with the highest value (Table 3). In general, cultivars had good leaf health.

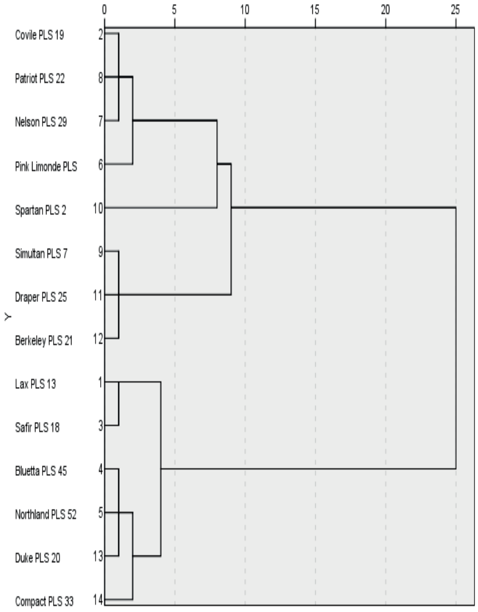


Figure 2. Cultivars grouped clustered by the morphological parameters

Based on the morphological parameters, cultivars were analyzed in clusters (Figure 2). According to the obtained dendrogram, four common groups were obtained. The first group included Coville PLS 19, Patriot PLS 22, and Nelson PLS 29; the second group Simultan PLS 7, Draper PLS 25, and Berkeley PLS 21; the third group Lax PLS 13, and Safir PLS 18; and the fourth group Bluetta PLS 45, Northland PLS 52 and Duke PLS 20.

CONCLUSIONS

The first data about the hybrid serie obtained from mother plants by free pollination are valuable for further research. A database of images and data was obtained and consist as a useful base for an extended tool in performing phenotyping research with the help of digital tools.

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ECOLOGICAL APPLE CULTURE IN ROMANIA - CULTIVATION AND CULTIVARS

Viorel STOICA, Dorel HOZA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Abstract

In the last decade, European consumer demand for organic or ecological products has increased. Ecological agriculture has the potential to reduce negative impacts on humans and ecosystems, but its productivity compared to conventional agriculture remains a controversial issue. Consumers began to look for safer and better controlled fruits, produced in a more ecological environment. Organically produced fruits are considered to satisfy consumer demands while having a favorable impact on the environment and human health. European Union guidelines on organic production prohibit the use of synthetic products (fertilizers and plant protection methods). The principles for organic farming are similar in different European countries and the permitted inputs are regulated by law. In this paper, we proposed to present principles and rules in ecological fruit growing sector, as well as fertilizers and varieties recommended for apple culture in an ecological system in Romania.

***Key words**: apple, ecological system, rules, cultivars, fertilizers.*

INTRODUCTION

The definition and state of ecological agriculture

"Ecological agriculture" is a term protected and assigned to Romania by the EU to define this agricultural system and is similar to the terms "organic agriculture" or "biological agriculture" used in other member states. For example, the term organic is used in Great Britain, Cyprus, Ireland, Malta, the term biological in France, Italy, Belgium, Greece, Luxembourg, Austria, the Netherlands, Portugal, and the term ecological in Denmark, Sweden, Lithuania, Poland, Spain, Romania, Slovenia, Hungary, Germany, etc. (Butac et al., 2021).

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) define organic agriculture as an "integrated system of managing the agricultural production process, which contributes to supporting and strengthening the resilience of the agro-ecosystem, including biodiversity, biological cycles and soil biological activity" (Butac et al., 2021).

According to the most recent Research Institute of Organic Agriculture (FiBL) data (2019), organic agriculture is practised in 187 countries, and 72.3 million hectares of agricultural land were managed organically by at least 3.1 million farmers. The regions with the organic agricultural land areas are Oceania (35.5 million hectares, 50% of the world's organic agricultural land), Europe (16.5 million hectares, 23%), Latin America (8.3 million hectares, 11%), Asia (5.9 million hectares, 8%), North America (3.6 million hectares, 5%) and Africa (2 million hectares, 3%) (Figure 1) (Willer et al., 2021).

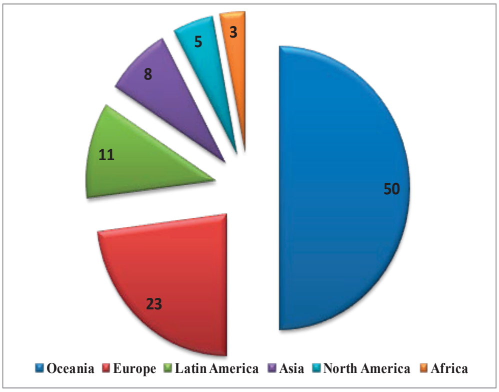

Figure 1. Distribution of organic agfricultural land by region, 2019 (surce: FiBl survey, 2021)

Worldwide, in 2016, fruit tree species from the seed and stone fruit group in the ecological system occupied an area of 254,600 hectares, berries 56,443 hectares (Butac et al., 2021), and nut crops occupied an area of 574,069 hectares.

In Europe, Spain, France, Italy, Germany and Switzerland are the countries with the largest orchard area in the ecological system.

Thus, Germany is the country with the oldest tradition in organic agriculture, at the level of 2017, 15% of the total production of fruits (apple, pear, plum, sweet and sour cherry, apricot, peach, quince, walnut) being produced in an organic system.

In Italy, in the South Tyrol region, 41,000 tons of apples are produced annually, of which 10% are organically grown. Under the slogan "in harmony with nature", the farmers of this region formed an association of apple producers in an integrated system, AGRIOS, since 1988.

In Switzerland, the pioneer of organic farming in the world, apples are the most sold fruits. At the level of 1999, there were 366 farmers, who owned 213 ha of apple orchards, of which 70-80% produced organically.

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).

Principles of ecological agriculture (EA) (Kienzle and Kelderer, 2017; Butac et al., 2021):

1. The principle of health: EA must ensure and improve the health of the soil, plants, animals and people. Ecological farming is intended to produce healthy food with superior nutritional quality that prevent disease and maintain well-being.
2. The principle of ecological: EA must achieve an ecological balance by organizing agricultural production systems, managing habitats and maintaining genetic and agricultural diversity.
3. The principle of fairness regarding the environment and living conditions. Fairness is expressed through respect, justice and consideration.
4. The principle of management that EA must be managed in a prudent and responsible manner to protect the health and well-being of current and future generations, as well as the environment. EA must prevent the occurrence of major risks by adopting appropriate technologies.

The main rules of ecological farming (Kienzle and Kelderer, 2017; Butac et al., 2021):

1. Environmental protection. EA must reduce or eliminate the use of synthetic or natural products that destroy useful organisms in the soil, diminish non-renewable resources and diminish the quality of water, air and agricultural products.
2. Maintaining and increasing soil fertility. EA through specific practices aims to intensify the activity of microorganisms in the soil, in order to increase its fertility.
3. Respect for consumers' health. By practicing EA, the aim is to obtain quality agricultural products, without pesticide residues, with a balanced content of proteins, carbohydrates, lipids, vitamins, organic acids and mineral salts.
4. The ecological farm must be a balanced unit being a component of the ecosystem.
5. Recycling of materials and resources inside the farm.
6. Maintaining the biodiversity of the agricultural ecosystem.
7. Cultivating plants in harmony with natural laws.
8. Obtaining optimal and not maximum productions. Maximum productions are achieved in most cases with an abusive use of resources and environmental degradation. Ecological agricultural systems aim to obtain optimal productions, under the conditions of environmental protection, agricultural products and conservation of non-renewable resources.
9. The use of technologies suitable for the ecological farming system.
10. Preserving the integrity of ecological agricultural products, from their production to marketing.

The legal framework and normative acts regarding ecological fruit growing

The European Commission has established rules for each type of activity through Regulation (EU) 848/2018 regarding production and labeling of organic products, Regulation (EC) no. 834/2007 and Regulation (EC) 889/2008 which provide application of appropriate management of biological processes based on ecological systems using natural resources (Kienzle and Kelderer, 2017).

In Romania, the ecological farming system is regulated by the following normative acts:

- Order 464/2019 regarding the import of ecological products from other countries;
- MADR Order 181/2012 and 954/2016 regarding the organization of the inspection and certification system in ecological agriculture;
- MADR Order 737/2014 regarding the registration of operators in ecological agriculture;
- Decision 131/2013 regarding the labeling of ecological products;
- Order 51/2010 regarding the authorization of imports of ecological agri-food products from other countries;
- Regulation 889/2008 regarding production and labeling of ecological products;
- Regulation 852/2004 regarding the hygiene of food products.
- Order no. 417/110/2002 regarding the labeling of ecological food products;
- Emergency Government Ordinance 34/2000 regarding ecological agri-food products.

Even if ecological agriculture has developed in many countries, there is a need for research activities, as follows:

• Creation of cultivars and rootstocks suited to ecological systems;
• Using of fertilizers and pesticides suited to ecological agriculture to improve soil fertility and maintain the health of plants, people and the environment.

Creation of cultivars and rootstocks suited to ecological systems

Appropriate cultivars and rootstocks are a key factor in development an orchard system (conventional and ecological).

Apple cultivars should show resistance to diseases, pests and abiotic factors such as frost or sunburn.

After the 1990s, many new cultivars from breeding programs around the world were tested and promoted as tolerant or resistant to one or more diseases.

In the last years, in the ecological fruit-growing sector and also in the market was introduce new scab-resistant cultivars with higher fruit quality, such as 'Topaz', 'Red Topaz', 'Dalinred', 'Santana', 'Ariwa' and 'Natyra' due to collaboration between breeder, growers and marketing organizations (Kienzle et al., 2016; Kienzle and Kelderer, 2017). These cultivars have the Vf scab resistance gene.

The breeding strategies for creation of new cultivars are based on two new approaches:

1. Combining of scab resistances with powdery mildew (*Podosphaera leucotricha*) resistance and fire blight (*Erwinia amylovora*) tolerance (Kellerhals et al., 2016).
2. Using in artificial hybridization of genetically distant cultivars. Avoidance of inbreeding is also an important issue (Ristel et al., 2016).

The evaluation of varieties from pomological collections for susceptibility to different diseases is crucial for both approaches (Kellerhals et al., 2016; Lateur et al., 1994.

In Romania, during the last 20 years, breeding objectives have mainly focused on fruit quality and disease resistance. The apple breeding programs for resistance to scab (*Venturia inaequalis* Cke.) have mostly concentrated on Vf gene originated from *Malus floribunda* 821 (Militaru et al., 2019).

Thus, from the breeding program carried out in Romania, a number of 65 new apple cultivars resulted, the great majority being resistant or tolerant to specific diseases.

Many of these cultivars have been propagated and spread in commercial plantations in our country, being highly appreciated by growers and consumers, such as 'Romus 3', 'Romus 4', 'Rebra', 'Rustic', 'Valery', 'Remar', 'Luca', etc. (Table 1).

Table 1. Apple cultivars recommended for organic farming

No.	Cultivars	Short description
1	Romus 3	Resistant to scab; summer cultivar; fruit: red color, ovoid, very good taste, highly appreciated by consumers and growers.
2	Romus 4	Resistant to scab; autumn cultivar; fruit: red color, flattened spherical, good taste.
3	Rebra	Resistant to scab; winter cultivar; fruit: red color, spherical, good taste.
4	Rustic	Resistant to scab; winter cultivar; fruit: red color, spherical, good taste.
5	Rumina	Resistant to scab; winter cultivar; fruit covered with rust on the surface, with good storage.
6	Topaz and Red Topaz	Autumn varieties; resistant to scab; fruit: yellow color with red stripes in the Topaz cv., and red color in the Red Topaz cv.; sweet-sour taste.

No.	Cultivars	Short description
7	Choupette® Dalinette	Winter cultivar; resistant to scab; fruit: red color; sweet taste; good storage capacity.
8	Natyra	Winter cultivar; resistant to scab, but sensitive to powdery mildew and bacterial cancer; fruit: dark red epidermis, crispy pulp; good storage capacity.

Using of fertilizers suited to ecological agriculture to improve soil fertility

In ecological orchards, two categories of fertilizers are allowed - organic fertilizers (manure, urine, compost, green fertilizers and plant residues) and mineral natural fertilizers (rocks and mineral products) (Toncea et al., 2016) (Table 2).

Table 2. Fertilizers used in ecological fruit growing

No.	Fertilizers tip	Short description and your role
1	Manure	Mixture of solid and liquid manure from animals; it contains many nutrients necessary to restore soil fertility and plant nutrition.
2	Urine and manure wort	Liquid which are used both as basic and foliar fertilizer.
3	Poultry manure	Fast-acting fertilizer with almost double the N, P and K content comparative with manure.
4	Compost	Organic fertilizer resulting from the controlled fermentation of a mixture of organic waste, such as vegetable waste, and urban sludge, etc.
5	Green fertilizers	The plants used are peas, lupine, clover; when the plants are in full growth; they are chopped and inserted under the soil.
6	Natural mineral fertilizers	In organic agriculture, only natural products are used as mineral fertilizers, not industrial ones; these materials (natural rocks, ores) in addition to a dominant nutritional element (phosphorus, potassium) also contain other elements necessary for plant nutrition.

CONCLUSIONS

Considering the fact that the agro-food market is dominated by fruits from conventional orchards, heavily chemical, by food additives used to improve the color, flavor and structure of food and their preservation, the organic products market can contribute to the protection of the environment and the health of generations current and future.

Researchers should develop a set of measures such as: breeding and introduction into culture and market of cultivars with durable resistance to pathogens; improving technological sequences for soil and vegetation management with the aim of increasing yielding capacity and fruit quality; improving technological sequences to ensure plant health.

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RIPENING AND POSTRIPENING OF ASIMINA (*ASIMINA TRILOBA* L. DUNAL) FRUITS (REVIEW)

Andrei Florin TABACU[1], Ioana Mihaela MIHĂLCIOIU[1], Luis OLTEANU[1], Ana Cornelia BUTCARU[2], Florin STĂNICĂ[1, 2]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Centre for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

Pawpaw or Northern banana (Asimina triloba L. Dunal), is part of the Annonaceae family, although it is a temperate fruit tree species. The pawpaw fruit is an exciting new food option for people looking for new, balanced food choices. One hundred grams of pawpaw pulp delivers 80 calories and contains 1.2 g of proteins and 1.2 g of total fat. Studies have shown that during ripening, loss of firmness is extremely rapid. This trait may be the biggest obstacle to developing a broader market, as handling without injury is difficult. Cold storage of pawpaw seems limited to four weeks at 4°C. Cold storage for longer than four weeks caused cold injury symptoms such as black discoloration, rapid loss of firmness, impaired respiration, tissue acidification, decreased antioxidant content, reduced volatile ester production, and development of off-flavor volatile compounds. This paper aims to review pawpaw ripening and postripening characteristics under local conditions.

Key words*: firmness, cold storage, cold injury, paw-paw.*

INTRODUCTION

Pawpaw or Northern banana (*Asimina triloba* L. Dunal), is part of the Annonaceae family, although it is a temperate fruit tree species.

The pawpaw fruit is an exciting new food option for people looking for new, balanced food choices.

This paper aims to review pawpaw ripening and post-ripening characteristics under local conditions.

RESULTS AND DISCUSSIONS

Asimina or paw-paw has a specific profile regarding nutritional values.

One hundred grams of pawpaw pulp delivers 80 calories and contains 1.2 g of proteins and 1.2 g of total fat. At the same time, 18.8 carbohydrates, 2.6 dietary fiber, 1g vitamin A, 30.5 vitamin C, 0.8 mg/100 g fw) thiamin, 6 mg/100 g fw riboflavin, 6.5 mg/1000 g fw niacin. For the minerals, in 100 g fw, there were 9.9 g K, 7.9 g Ca, 5.9 g P, and 35.9 g. Mg, 56 g Fe, 6.7 g Zn, 22.2 g Cu, and 74.3 g Mn (Paw-paw, 2023).

The fat composition of pawpaw fruit consists mainly of unsaturated fatty acids such as palmitoleic (5.8-10.2%), oleic (23.2-42.0%), linoleic (8.0-12.0%), and linolenic (14.0-24.4%) (Peterson, 1982).

For the essential amino acids, in 100 g fw, there were 21 g histidine, 70 g isoleucine, 81 g leucine, 60 g lysine, and 51 g phenylalanine (Paw-paw, 2023).

Despite the rancidity problem, pawpaw-derived products as carbohydrate-based, fat-reducing agents in baked food formulations have been studied (Wiese & Duffrin, 2003).

More research suggested that pawpaw fruit pulp had the potential to be added to various consumer goods to add increased nutritional benefits or flavor enhancement (Brannan et al., 2012).

Pawpaw fruits are fragrant and nutritious, with a unique aroma and flavors like a combination of banana, mango, and pineapple (Duffrin & Pumper, 2006).

Literature data consider pawpaw pulp as a good source of polyphenols (ranging from 22.13 to 37.36 mg GAE per g) and apples, oranges, grapes, and strawberries (Brindza, 2019).

In particular, the predominant polyphenolic compounds were three phenolic acids, protocatechuic acid hexoxide, p-coumaroyl hexoxide, and 5-O-p-coumaroylquinic acid, and flavanols, particularly (−)-epicatechin, B-type procyanidin dimers and trimers (Brannan et al., 2015).

There has been interest in the pawpaw because of its tropical scent and flavors, leading to its niche use. Recent research has shown the pawpaw to have a fermented yeasty smell, with many of the natural scents coming from alcohol or alcohol derivatives (Goodrich et al., 2006).

Volatile methyl esters increase as the fruit matures. As the pawpaw ripens, fruity aroma, sweet aroma, melon-like aroma, and fermented aroma intensities increase, and cut-grass (green) intensity decreases (McGrath & Karahadian, 1994).

However, flavor and aroma vary among pawpaw varieties, with some fruits displaying complex flavor profiles that have not been described. Pawpaw fruit ripening is characterized by increased soluble solids concentration (up to 20%), flesh softening, increased volatile production, and, in some genotypes, a decline in green color intensity (McGrath & Karahadian, 1994).

Determining the levels of phytochemicals in pawpaw may benefit attempts to commercialize the fruit. Two studies have reported levels of antioxidant compounds in pawpaw pulp. One group reported that phenolic content and antioxidant capacity in fruit from two pawpaw cultivars tended to decrease with ripening (Kobayashi et al., 2008).

The amount of total phenolics in pawpaw pulp was not affected by the level of ripeness. However, the level of total flavonoids was 40% higher in underripe pawpaw than ripe, which was 12% higher than in overripe pulp. The level of total phenolics reported in pawpaw (9.2 μmol gallic acid equivalents per gram fresh fruit) was similar to several commercially important *Annonaceous* fruits, including soursop/guanábana/graviola (Harris & Brannan, 2009).

The preliminary analyses indicated that firmness declined due to the action of at least four enzymes: polygalacturonase, cellulase, pectin methylesterase, and endo-ß-man-nanase (Koslanund, 2003).

Pawpaw fruit has traditionally been harvested from native plant stands and small orchards for immediate sale and consumption. Other than the decline in fruit firmness, there are no. Obvious indicators of fruit ripening. This lack of a good harvest index is also a problem with the related cherimoya (Merodio & De la Plaza, 1997).

The fruits on a single tree do not ripen within proximity in time to one another. An extended harvest period of two weeks or more from a tree is not uncommon. The protracted harvest may be partly due to the staggered spring bloom period, up to two weeks or more. Each fruit cluster develops from an individual flower, and fruit within a cluster often ripen at different times. Cultivar variation in harvest date also exists, with early to late season cultivars ripening over 4 to 6 weeks. Currently, multiple harvests from one tree are conducted to obtain high-quality fruit. Because a decline in firmness is the main indicator of ripening, this requires repeated visits to and touching individual fruit, which is very laborious. A once-over harvest from a tree is not feasible (Miller, 1989).

Since pawpaw exhibits an ethylene climacteric, it too may be susceptible to ethylene treatment to induce ripening and treatments to affect ethylene production or action. It was found that fruit harvested immature did not ripen, even if treated with ethephon at 1000 mg•L^{-1}, but using commercially available growth regulators to manipulate pawpaw ripening warrants further study. Postharvest application of 1-methylcyclopropene, an ethylene action inhibitor, has recently been used to successfully slow the ripening of climacteric species and may help do the same with pawpaw (Fan et al., 1999).

Although germplasm selection and breeding have led to more pawpaw cultivars, two main obstacles to market interest development, the fruit's rapid postharvest perishability and the absence of harvest synchronization within and among trees, have not yet been overcome.

Once riped, pawpaw fruit is marketable for only 3-4 days when held at room temperature (Archbold & Pomper, 2003).

When fully ripe, the fruit must be handled with care since its thin skin and highly soft pulp expose it to bruises and other physical damages

(Peterson, 1991; Archbold et al., 2003; Archbold & Pomper, 2003)

Pawpaws fail to complete the ripening process if harvested too early (Archbold, personal communication).

Ripe pawpaw fruit could be cold stored at 4°C for 4 weeks with minor changes in fruit quality (Archbold et al., 2003; Archbold & Pomper, 2003).

Like other climacteric fruits, pawpaw ripening is characterized by increased ethylene production and respiratory activity (Archbold & Pomper, 2003; Koslanund et al., 2005). Single peaks of each were generally detected three days after harvest.

Mean ethylene on a fresh weight basis were 4.7 and 7.6 $\mu g\ kg^{-1}h^{-1}$, and mean respiratory (CO_2 production) maxima on a fresh weight basis were 220 and 239 $mg \cdot kg^{-1} \cdot h^{-1}$ in 1999 and 2001 harvests, respectively.

Ethylene and respiration peak values in pawpaw were similar to those reported for sugar apple and cherimoya. However, ethylene values were significantly lower and respiration values significantly higher than the values reported in other climacteric fruits such as apple, which at harvest produces 10-100 $\mu g\ C_2H_4 \cdot kg^{-1} \cdot h^{-1}$ and 5-10 $mg\ CO_2 \cdot kg^{-1} \cdot h^{-1}$ (Kader, 2002).

Figure 1. Unripe pawpaw fruits (source: own data)

Harvested fruits are still living organs; hence, even though detached from the plant, they continue to exchange gas with and lose water to the environment.

Since the connection with the mother plant has been cut, the respiratory substrate and water losses that occur cause permanent changes in fruit composition (Burdon, 1997).

Many preharvest and postharvest factors, such as genetics, cultural practices, maturity at harvest, and postharvest handling techniques, influence the composition and quality of fruit by the time it reaches the consumer (Archbold et al., 2003).

Figure 2. Ripe pawpaw fruits (source: own data)

Cold storage delayed the ripening of fruit and significantly delayed firmness loss. However, firmness declined rapidly upon removal from cold storage, accompanied by a rise in ethylene production and respiration.

Like cherimoya (Merodio & De La Plaza, 1997), cold-stored pawpaw fruit exhibited a higher ethylene maximum than fruit ripened after harvest (Archbold et al., 2003).

Preliminary observations indicated that more extended storage periods resulted in external and internal black discoloration of the fruit (Koslanund, 2003), possibly symptoms of cold injury similar to other *Annonaceae* like cherimoya (Martinez-Tellez & Lafuente, 1997).

Figure 3. Harvested fruit (source: own data)

In the research of Galli (2007), fruit were cold stored at 4°C for 0 (harvest) 2, 4, 6, or 8 weeks. Measurements for ethylene production were collected daily for three days after beginning bench ripening and were expressed as µg C_2H_4/kg fw/h. Respiration was determined and expressed as mg CO_2/kg fw/h.

The highest ethylene production was recorded two weeks after harvesting by the Middletown variety (7.00 µg C_2H_4/kg fw/h). Only PA Golden and Taytwo fruits stayed mold–free until the end of the eight weeks of cold storage.

'9-58' and Middletown fruits were the firmest (32 N and 27 N, respectively) in 2004, and Taytwo and Middletown fruit were the firmest (28 N and 26 N, respectively) in 2005.

After two weeks of cold storage, the overall number of fruits with firmness higher than 15 N decreased from 71% (at harvest) to 19%.

Taytow recorded the highest amounts of glucose and sucrose in the 8th week and the most significant amount of starch when the fruit was unripe.

Taytwo pawpaw fruit pH significantly increased with ripening after harvest and 4 or 6 weeks of cold storage. After eight weeks of cold storage, the fruit pH had significantly decreased.

Headspace volatile profile composition of pawpaw cultivars at harvest was determined. At harvest, when fruit was considered in the early ripening stage, Wilson produced a significantly higher amount of total volatile compounds, 10-fold or more, than the other cultivars similar to one another.

During bench ripening, the rise in volatile production was principally linked to an increased emission of hexanoate and octanoate esters for Taytwo. The concentration of hexanoates increased by more than 700-fold during ripening. The major volatiles detected were (following retention time) ethyl alcohol (EA), ethyl acetate (EAC), ethyl butanoate (EB), methyl hexanoate (MH), ethyl hexanoate (EH), methyl octanoate (MO), octanoic acid (OA), ethyl octanoate (EO), ethyl decanoate (ED), ethyl propionate (EP), hexanoic acid (HA), methyl butanoate (MB).

Regarding enzyme activity, in the Taytwo cultivar, AAT activity was detected at an early ripening stage. ADH activity in Taytwo fruit was almost 50- and 3 times lower than ADH activity measured in apples and tomatoes at harvest, respectively.

LOX activity at harvest was comparable to that reported for apples and strawberries. As with the ADH activity above, the variability of the LOX data was high.

Alcohol acyltransferase (AAT), alcohol dehydrogenase (ADH), and lipoxygenase (LOX) activity of Taytwo fruit at 4 and 72 h after harvest or after 2, 4, 6, or 8 weeks of cold storage were determined. Enzyme activities were expressed as mU/mg protein.

The highest total glutathione and GSH concentrations were measured in fruit bench ripened for 72 h after harvest and after 2 and 4 weeks of cold storage.

Content of total glutathione, oxidized glutathione (GSSG), reduced glutathione (GSH), and glutathione reductase (GR) activity in Taytwo cultivar one week before commercial harvest (unripe), at harvest, and after 2, 4, 6 and 8 weeks of cold storage was also determined. Measurements were collected after 4 and 72 h of bench ripening.

The ascorbate content of Taytwo fruit at harvest was similar to that reported in other research. The highest total ascorbate concentration was in fruit at harvest. Values had significantly dropped by two weeks of cold storage, and there were significant declining trends over storage time when measured at 4 or 72 h. However, bench ripening did not affect total ascorbate.

As for total antioxidant activity during storage and ripening, no differences were found among values one week before commercial harvest (unripe), at harvest, or after 2, 4, 6, or 8 weeks of cold storage, irrespective of 4 or 72 hours of bench ripening. Even though cold storage did not affect total antioxidant content, phenolic concentration varied with storage length.

Carotenoid content varied among pawpaw cultivars. Wilson cultivar had the highest carotenoid content, and '8-20' had the lowest. Fruit of carotenoid-rich cultivars had the most intense pulp color, and carotenoid-poor cultivars had moderate pulp color.

PA Golden cultivar was used to investigate the possible modification of carotenoid content during cold storage. Carotenoid content at harvest was significantly higher than in unripe fruit, but no differences were observed between fruit at the beginning and the end of bench ripening.

Brannan (2015) conducted a study and determined the PPO activity of five pawpaw cultivars. Significant Polyphenol oxidase activity was observed in Sun Flower > Green River Belle > Susquehanna ≥ Wild ≥ Sue. All cultivars exhibited pulp pH in the range of 5.9-6.3. Previous research has shown that pawpaw pH exhibits maximum activity at pH 7.0, while pH 6-7 range values exhibit high activity (Fang et al., 2007; Brannan, 2015). In addition to having the lowest PPO activity, variety Sue had the lowest sugar content (15%) compared to the other varieties. However, there was no clear trend concerning sugar content and PPO activity in the other varieties (Brannan, 2015).

In another study, GRB was the only variety in common with both of the varieties analyzed in each of the two phases of this study. The pH values of the varieties ranged from 6.1 to 6.8, which is in the high range of pawpaw PPO activity. The PPO activity of GRB was in good agreement between the two phases of the current research, exhibiting PPO activity (ΔABS/min/g protein) of 5.31 and 5.36. PPO activity among the 12 variations showed that six varieties (T2, RG, NC1, OL, RAP, and GRB) exhibited PPO activity statistically higher than QD and LF. The other four varieties (SAAZ, SHEN, ATW, IXL) exhibited PPO activity that was not significantly different from each other or QD and LF. However, they were significantly lower than T2, RG, and NCl (Brannan, 2015).

The antioxidant activity of *Asimina triloba* genotypes evaluated by the DPPH method ranged from 2.84 (AzT-01) to 7.04 mg TEAC. g^{-1} (AzT-04). The variation coefficient (31.77%) in all the genotypes tested confirmed the degree of mean variability of parameters. The antioxidant activity evaluated by the molybdenum-reducing antioxidant power varied from 97.25 (AzT-06) to 275.41 mg TEAC.g^{-1} (AzT-03). The degree of mean variability of parameters was confirmed by the variation coefficient (35.07%) in tested genotypes (Brindza et al., 2019).

The total polyphenol content in *Asimina triloba* genotypes ranged from 22.13 (AzT-05) to 37.36 mg GAE.g^{-1} (AzT-02). The variation coefficient (16.87%) confirmed the high variability of the parameter. The differences between the present and previously conducted studies may be attributable to the plant's geographical origin as well as the different methods of extraction. The flavonoid content varied from 15.10 (AzT-05) to 32.02 mg.g^{-1} QE (AzT-02). The variation coefficient (25.39%) supported this parameter's observations of high variability. The total phenolic acid content was found to vary significantly among the various *Asimina triloba* genotypes, possibly due to their different botanical and regional origins. The mean total phenolic acid of the studied fruit genotypes was 25.16 mg.g-1 CAE, with the highest phenolic acid recorded by genotype AzT-02 at 32.02 mg. g^{-1} CAE, indicating its superior antioxidant potential. The variation coefficient (25.39%) supported this parameter's observations of high variability (Brindza et al., 2019).

The analysis of the ripening behavior of 10 different pawpaw cultivars (1-7-2, 8-20, 9-58, Middletown, PA Golden, Shenandoah, Taytwo, Taylor**,** Wells, and Wilson) over two seasons showed that all genotypes ripened similarly after harvest. For fruit held for up to three days at room temperature, CO_2 production ranged from 48 to 174 mg CO_2/kg fw/h, ethylene production ranged from 0.2 to 2.7 µg C_2H_4/kg fw/h, and firmness decreased by 30-50%. In both harvest years, ethylene and CO_2 peaks were generally detected within 48 hours from harvest. No differences in the cold storage response were found among the different genotypes. After cold storage, ethylene production ranged from none detected to 28 µg C_2H_4/kg/h, respiration ranged from 0 to 214 mg CO_2/kg fw/h, and firmness declined as the storage period increased (Galli, 2007).

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INFLUENCE OF PHOTOSELECTIVE PROTECTIVE NETS ON THE SENSORY CHARACTERISTICS OF FRUITS OF THE FLORINA APPLE CULTIVAR

Sava TABAKOV[1], Petya IVANOVA[2], Galya DOBREVSKA[1], Anton YORDANOV[1], Manol DALLEV[1]

[1]Agriculture University - Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria
[2]Institute of Food Preservation and Quality, Plovdiv, Bulgaria., 154 V. Aprilov Blvd, Plovdiv, Bulgaria

Corresponding author email: manol_dallev@abv.bg

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 used nets 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 Florina apple fruits was studied in an orchard with a support structure located in Northern Bulgaria.
The sensory evaluation of the Florina cultivar shows that the fruits under the most commonly used black net in orchards in terms of taste, aroma and consistency do not differ significantly from those grown under yellow and red coverings. Only the influence of different types of nets on the appearance and skin colour of the fruits of the Florina apple variety has been statistically proven.

Key words*: Florina, photoselective nets, sensory analysis, appearance, colour, taste, 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 producing.
Net systems are usually used to protect orchards from hail. Nowadays the systems 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).
According to Meena et al. (2016), photoselective nets are able to scatter light and this results in improved solar radiation utilization efficiency of fruit plants. This would change metabolic processes and positively affect flower bud formation, flowering intensity and ultimately better retention and development of quality fruits (Sivakumar et al., 2017).
The relationship between fruit trees and environmental conditions affecting fruit quality differed for open areas and areas covered with flower anti-hail nets. The reason for this is the change of the microclimate under the nets, which affects differently the coloring of the fruit surface and the influence of chlorophyll on the quality of the fruit flesh (Bosco et al., 2015; Brglez Sever et al., 2015; Dussi et al., 2005; Reay et al., 1998).
The characteristics determining the quality of the fruit flesh - firmness, ripeness, sugar content and acidity - are less affected in the studies on the influence of the anti-hail nets, compared to the parameters determining the appearance of the fruit - size and color. The flesh firmness varies according different types of nets. Differently colored photoselective nets stimulate variable responses to fruit flesh substances in different fruit species. Giaccone et al. (2012) reported that fruits grown under white nets had higher sugar content than those grown under red nets. Many authors did not find differences in the acidity of the fruits of trees covered with differently colored nets (Ordóñez et al., 2016).
The quality of the fruits can also be reduced by the presence of sunburn. They can adversely affect 10-50% of apple fruit yield (Wünsche et

al., 2001; Kalcsits et al., 2017). Moreover, some cultivars are considered very susceptible to this type of damage (Dussi et al., 2005). According to some authors, black nets are more effective than white nets in reducing these damages (Amarante et al., 2011).
From what has been summarized so far, it is clear that the nets used in fruit growing, in addition to protection, can also be designed to screen spectral bands of solar radiation and transform part of the direct light into diffuse, contributing to increasing the quality of fruit production from various fruit species and their cultivars. This puts intensive fruit growing at a higher level in its development. Studies on the influence of photoselective nets on the physico-chemical and sensory quality characteristics of fruits in apple cultivars are insufficient. The aim of the study is the accumulation of knowledge and information to evaluate the influence of photoselective nets on the sensory characteristics of fruits of the Florina apple cultivar, based on the overall sensory evaluation and the average evaluations of the appearance, color, consistency, aroma and taste indicators.
The apple cultivar Florina was introduced in Bulgaria in 1977. The cultivar is early bearing and productive. It is resistant to the economically important diseases scab and powdery mildew. Harvest maturity occurs at the end of September (Lichev et al., 2012).

MATERIALS AND METHODS

The physico-chemical and sensory quality characteristics of apple fruits of Florina cultivar budded on M9 T337 rootstock and grown in an orchard located in Northern Bulgaria were monitored. The planting density is 250 trees per decare. A sod-mulch system is applied to maintain the soil surface and trees are drip irrigated. Anti-hail net system has been built in black, white, yellow and red colouring.
The application of sensory analyzes is to describe raw materials/products using a language that is close to that of the consumer, to characterize them in an objective way qualitatively and quantitatively, or to explain the overall profile and perceived quality of the food (Murray et al., 2001; Seppä et al., 2012; Swahn et al., 2010). For these reasons, sensory analyzes require scientific expertise and appropriate laboratories equipped to perform sensory tests (ISO 8589:2007).
The analyzes were carried out in the Food Testing Laboratory at the Institute of Food Preservation and Quality - Plovdiv 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; color - 0.20; consistency - 0.20; taste - 0.20 and aroma - 0.10.
Five fruits per variant were obtained on the day of harvest and stored for one day at 20°C ± 5°C until the sensory evaluation. When assessing the quality of Florina apple friuts, 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 Regulation (EU) No. 1580/2007 of the Commission of December 21, 2007 for determining 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 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. The weight of the fruit was determined with a laboratory technical balance A200 S, in grams (g). Fruit height and width were measured with a digital calliper, values were expressed in millimetres (mm). The data are 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 professionals to interpret the degree of sensations of taste, sweetness and acidity.

Table 1. Description of the studied sensory indicators of apple fruits, cultivar Florina

Parameters	Description
Appearance - Fruit shape;	Flat-conical to globose-conical, asymmetric with weak ribbing or asymmetric shape. Handle fossa - wide, deep with gentle gray-brown rust, fairly regular. Calyx fossa - medium to large, irregular, with small ridges along the rim
- Size	Medium to large, according to the size in Commission Regulation (EU) No. 1580/2007 of December 21, 2007, the fruits belong to group L - Section diameter: = extra-65mm/70mm; = 1st quality - 60 mm/65 mm. - Weight: = extra 110 g/140 g = 1st quality - 90 g/ 110g.
Skin colour	General surface of the fruit is a mixed red coloration with a pale green to pale yellow base color and red fuzzy or streaked covering color, with large gray-brown rusty dots or yellow green ground color, almost entirely covered with bright red to violet red streaks and fuzzy color with numerous white subcutaneous dots or yellow green main color, the roof fuzzy in stripes red to dark red covering almost the entire fruit, according to Commission Regulation (EU) No. 1580/2007 of December 21, 2007, the fruits belong to group B-varieties with mixed red coloration or range of coloration for : = extra 1/2; = 1st quality- 1/13; = 2nd quality -1/10.
Strength of fruit skin	Firm, medium-thick, slightly greasy, with a waxy coating and with large gray (brown) rusty dots or numerous white subcutaneous dots.
Colour of fruit flesh	White to cream
Consistency	Tender, crispy, juicy
Taste	Sweet, slightly acid
Aroma	Slightly aromatic

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 program, Microsoft Excel.

RESULTS AND DISCUSSIONS

Figure 1 shows the average evaluation of the experts for the appearance of the fruits (shape, uniformity, size, background scin color and surface defects). The committee of experts evaluated the appearance of Florina apple fruits from all growing variants with scores from 3.35 for fruits grown under the white net to the maximum score of 4.90 for fruits under the yellow net (Figure 1).

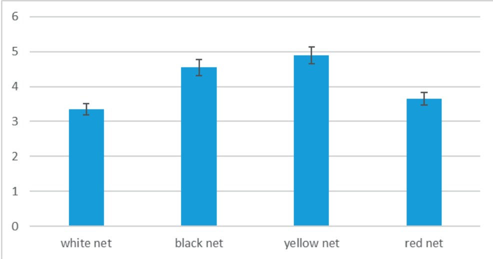

Figure 1. Appearance of fruits of cultivar Florina grown under different photoselective nets

The description of the appearance indicator characteristic of the cultivar is that the fruits are not uniform, with a flat-conical to spherically-conical, asymmetrical shape, with an average 0. According to the description under Commission Regulation (EU) No. 1580/2007 of December 21, 2007, all apple fruits meet the requirements for "Extra" quality. With the maximum values for weight and overall average evaluation for appearance are the apple fruits grown under a yellow net respectively: 193.74 g and 4.90, followed by the fruits grown under the black net (overall evaluation - 4.55, weight - 140.99 g and section diameter - 82.40 mm). The apple fruits grown under the white net have the lowest values according to the measured indicators, respectively: overall rating - 3.35; weight - 128.45 g and diameter of the section - 65.46 mm.

The handle fossa is wide, deep with gray-brown rust, relatively regular and varies from d = 15.26 mm in fruits under the yellow net to d = 31.01 mm in fruits under the red net.

Calyx fossa is medium-sized, irregular, with small protrusions on the rim.

Photo 1. Apple fruits of Florina cultivar, grown under a yellow net

Photo 2. Apple fruits of Florina cultivar, grown under a black net

Photo 3. Apple fruits of Florina cultivar, grown under a white net

Photo 4. Apple fruits of Florina cultivar, grown under a red net

The experts defined the descriptions of the skin color of the apple fruits on the differently colored nets and gave an average rating above 4.0 (Figure 2). Fruits under the yellow, red and black nets have no statistically significant differences and their maximum rating is 4.8. According to Commission Regulation (EU) No. 1580/2007 of December 21, 2007, the apple fruits belongs to group B - cultivars with mixed red fruit coloring and correspond to "Extra" quality.

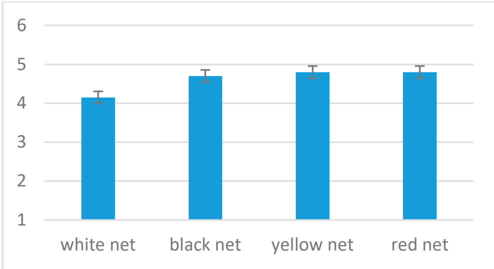

Figure 2. Skin color of fruits of cultivar Florina grown under different photoselective nets

Apple fruits of the Florina cultivar grown under yellow net (Photo 1) have a pale green main color and a red fuzzy colored integument with white dots and a wax coating.

Apple fruits of the Florina cultivar grown under black net (Photo 2) have a yellow main color, almost completely covered surface with a bright red to violet red fuzzy color, with numerous white subcutaneous dots, with medium to medium gray-brown rusty spots and a wax coating.

Apple fruits of the Florina cultivar grown under white net (Photo 3) have a yellow-green main color, almost completely covered with a bright red to violet red fuzzy color, with numerous white subcutaneous dots and a wax coating.

Apple fruits of the Florina cultivar grown under red net (Photo 4) have a pale green to pale yellow main and a red fuzzy covering color, with large gray-brown rusty spots and a wax coating.

The color of the fruit flesh of the studied variants varieties from white to cream.

Textural properties can be considered as the main factors responsible for fruit freshness and are related to consumer choice (Harker et al., 2008; Péneau et al., 2006; 2007). Texture consists of many different properties perceived by the human senses and its definition implies sensory evaluation (Bourne, 2002). Consumer preferences for a product are generally based on a combination of texture, taste and aroma (Daillant-Spinnler et al., 1996; Gatti et al., 2011; Harker et al., 2003).

For the consistency indicator, the experts determined that it was juicy, crunchy for all the tested variants and gave a rating above 4 (Figure 3).

The fruits under the yellow net have the highest rating (5.0), the fruits under the white net (4.3) have the lowest, and the rest of the fruits from the two versions of the black and red net have statistically indistinguishable ratings (4.6).

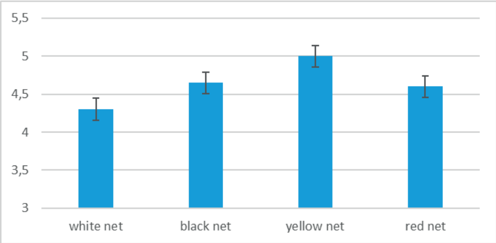

Figure 3. Consistency of fruits of cultivar Florina grown under different photoselective nets.

Chemical indicators that are used to determine fruit quality are concentration of soluble solids, total acidity, total sugars, but indicators such as appearance, surface defects, fruit firmness have a greater influence on the choice of consumers and traders and effectively making them unsellable.
All variants of tested apple fruits are sweet in taste. Fruits under the yellow net were rated by the experts with a maximum score of 4.8 and determined to be of moderate to weak acidity. Fruits under the red and black nets had a sweet-sour taste and statistically indistinguishable scores (4.6), and the fruits grown under the white net were defined as slightly sour and rated with the lowest score of 3.85 (Figure 4).

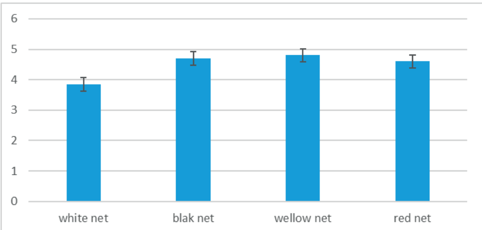

Figure 4. Taste of fruits of cultivar Florina grown under different photoselective nets

With the maximum percentages of dry matter, total sugars and sugar-acid ratio are the fruits grown under the red net, and with the lowest are the fruits under the black net, but these results are not directly related to the sensory evaluation given by the taste experts. Harker et al. (2002) also found a lack of correlation between measured soluble solids concentrations and expert perception of flavor intensity-sweetness and support the claim that the sensory parameter "taste" should remain a critical part of the evaluation of fruit quality, as sweetness intensity is one of the most important factors influencing consumer liking.
The multi-sensory character in defining an acceptable perception of apple fruit includes taste, smell and other sensory properties.
For the aroma indicator, the experts evaluated the fruits as aromatic the average evaluations are above 4.0, with the maximum evaluations of the apple fruits under the black, red, and yellow nets being statistically indistinguishable, and the fruits under the white net having the lowest evaluation (Figure 5). A positive linear relationship with an average coefficient of determination $R^2 = 0.54$ was established between the evaluations given by the experts for taste and aroma of the investigated variants of apple fruits of Florina cultivar.

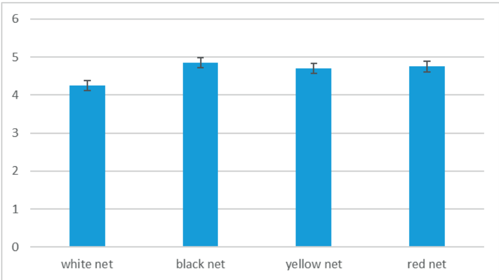

Figure 5. Aroma of fruits of cultivar Florina grown under different photoselective nets

From the conducted sensory analysis and the generalized average evaluations according to appearance, colour, consistency, taste and aroma indicators with the corresponding weighting coefficients, the total sensory evaluation presented in Figure 6.

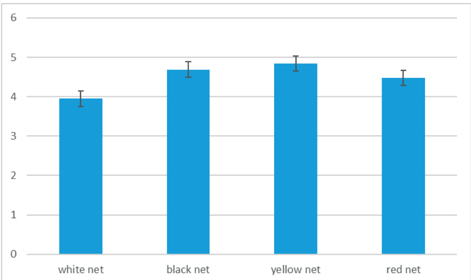

Figure 6. Summary sensory evaluation of fruits of cultivar Florina grown under different photoselective nets

The data show that apple fruits grown under the yellow net have the highest overall sensory score, followed by fruits grown under the black net. The fruits grown under the white net have the lowest sensory evaluation. A positive linear relationship was established with an average coefficient of determination between the overall tasting score and the aroma and color scores, respectively $R^2 = 0.67$ and $R^2 = 0.51$.

A study was conducted to evaluate the influence of photoselective nets on the sensory characteristics of apple fruits of the Florina cultivar based on the overall sensory evaluation and the average evaluations of the appearance, color, consistency, aroma and taste indicators.

The average ratings of apple fruits according to the studied indicators can be summarized as follows:

- the fruits grown under a yellow net have maximum ratings for all the investigated indicators;
- fruits under the red net have the highest percentages of soluble solids and total sugars;
- the apple fruits grown under the black net are rated as high as possible in terms of aroma indicator;
- apple fruits grown under a white net have minimal evaluations according to the indicators of appearance, consistency, taste and aroma;

From the analysis of variance conducted at a significance level $\alpha = 0.05$ to establish the influence of the color of the photoselective nets on the sensory indicators of the studied apple cultivar, it was found that the factor of the color of the net has an effect on the appearance and skin color of the fruits.

The summary sensory evaluations were statistically indistinguishable for fruit from the yellow, black and red nets, and statistically distinguishable for fruit under the white net, suggesting that Florina cultivar is suitable for growing under these differently coloured photoselective nets.

CONCLUSIONS

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POTENTIAL USE OF ENVIRONMENT-FRIENDLY *MAERUA ANGOLENSIS* EXTRACTS AS ALTERNATIVE TO SYNTHETIC NEMATICIDES IN RURAL SMALLSCALE CASSAVA PRODUCTION

Moses TIMANA[1], Nicholas MNYAMBO[1], Mmagadima SEBATI[1], Lerato KGOTSE[1], Rivoningo UBISI[1], Thobile MKHWANAZI[1], Mbokota KHOSA[2], Owen MBATYOTI[2], Dirk DE WAELE[3], Zakheleni DUBE[1]

[1]University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa
[2]Agricultural Research Council-Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa
[3]University of Leuven (KU Leuven), Willem de Croylaan 42, 3001 Heverlee, Belgium

Corresponding author email: mosestimana97@gmail.com

Abstract

Cassava is a crop with great potential in addressing the challenges of malnutrition, rampant in the African continent. In southern Africa, the crop has a minor status being produced mainly by smallholder farmers until recently. There is scant information on the effects of root-knot nematodes in cassava production. The aim of the study was to determine the contribution of Maerua angolensis extracts in the management of Meloidogyne incognita in two cassava cultivars (cv. 'Mbonisweni' and 'Mganduzweni'). Treatments were laid-out in a randomised complete block design (RCBD) under greenhouse conditions with five replications. Treatments of consisted of 0, Nemacur, 184; 368; 736; 1472; and 2944 kg/ha of M. angolensis. Both cultivars had nematode reproductive-factors and potential greater than one and no nematode effect were observed on plant growth of the two cassava cultivars. M. angolensis had equivalent effect on M. incognita populations as commercial nematicide, Nemacur. In conclusion, 'Mbonisweni' and 'Mganduzweni' cultivars were tolerant to M. incognita and M. angolensis plant extract have potential use as an alternative to commercial nematicides in cassava production.

Key words: *cassava, host-status, Meloidogyne incognita, Maerua angolensis, susceptibility.*

INTRODUCTION

Cassava cv. 'Mboniseni' has been reported to be tolerant to *M. incognita* (Timana et al., 2021). Since the crop is mainly grown in subsistence farming as an intercrop, the build-up of nematodes in the presence of the crop may affect the susceptible crops in the intercrop or a rotation with a susceptible crop (Kimaru, 2013). With the limited use of synthetic chemicals in subsistence farming, any build-up of nematodes is not desired as it creates a challenge for the next crop in rotation or an intercrop (Talwana et al., 2015). Currently, there are no reports of nematode management in the subsistence cassava farming sector, yet in the commercial sector, the management has relied mostly on the use of synthetic chemicals nematicides (Sithole et al., 2021).

Plant parasitic nematodes are thought to be responsible for 12.3% of agricultural losses, or USD 157 billion each year (Mendoza-de Gives, 2022). The environmental problems associated with the use of synthetic chemicals includes greenhouse gas pollution, mutagenicity, soil degradation, and depletion of aquatic life (Sithole et al., 2021). Some of the human health issues associated with synthetic chemicals include cancer, human endocrine disruption, infertility, and headaches (Jallow et al., 2017; Talwana et al., 2015). The use of synthetic nematicides in the subsistence farming sector is also challenged by the size of land under production, which affects the economies of scale and the knowledge of handling hazardous substances (Talwana et al., 2015). Misuse, health complications, and improper handling of synthetic chemicals especially in subsistence farming have been reported, with some smallholder farmers in Tanzania reported to be applying pesticides weekly in their farms without consideration of the pest injury levels (Talwana et al., 2015). In India, sprayers, and farmworkers exposed to chemicals such as methyl parathion were reported to suffer from cardiotoxic effects (Aktar et al., 2009).

Different plant extracts have shown the ability to manage root-knot nematode population in different crops over the years (Khosa et al., 2020a; Akpheokhai et al., 2012; Taye et al., 2012). Some of the widely assessed plant extracts include *Tagetes* species (Kalaiselvam & Devaraj, 2011), *Cucurbita maxima, Tithonia diversifolia, Azadirachta indica, Zanthoxylum zanthoxyloides* and *Datura metel* (Akpheokhai et al., 2012), and *Inula viscosa* (Ibrahim et al., 2016). *Maerua angolensis* and *Tabernaemontana elegans* have also suppressed *M. incognita* populations in tomato plants under glasshouse and field conditions (Khosa et al., 2020a). Two major disadvantages in the use of plant extracts have been the inconsistencies in the performance and phytotoxicity (Mashela et al., 2015). Mashela et al. (2015) postulated that these challenges are mainly caused by the use of too low or high dosages, affecting the level of adoption of plant extracts in several crops, in several countries.

The extensive work that has been done on the nematicidal effect of plant extracts with their root-knot nematode suppressive compounds, has led to the discovery of plant substances that are toxic to *M. incognita* (Khosa et al., 2021). These plant extracts with the ability to suppress root-knot nematode can either inhibit or repel second-stage juveniles to penetrate plant roots or restrict the movement of J2 after penetrating the root system, ultimately killing the nematode (Khosa et al., 2021). *Maerua angolensis* has been reported to have suppressed *M. incognita* populations in tomato plants under glasshouse and field conditions (Khosa et al., 2020a), but this has not been confirmed in cassava plants. Therefore, the present study aims to establish the potential of *M. angolensis* plant extracts in suppressing *M. incognita* and promoting growth in two locally produced cassava cultivars, cv. 'Mbonisweni' and cv. 'Mganduzweni'.

MATERIALS AND METHODS

Study area

The study was conducted at the University of Mpumalanga greenhouse (25°43'64'' S, 30°98'17'' E), Mbombela, Mpumalanga, South Africa with controlled temperatures of 25±5 ˚C.

Medicinal plant material collection and extract preparation

Stems and leaves of *M. angolensis* were collected from the Agricultural Research Council, Nelspruit. The botanical origin of the plants was identified and verified by a botanist from the South African National Biodiversity Institute (SANBI, Tswane), and the material were stored as code 3112000/PRE099594-0 (Khosa et al., 2020a). The *M. angolensis* plant parts were cut into 5 cm long parts and dried using an oven set at 52 °C for 4 days before being ground using Wiley mill and passed through a 1 mm sieve (Khosa et al., 2020a). The crudely milled plant material was kept in marked, air-tight glass vessels at 25°C in the shade until required for usage.

Preparation of nematode inoculum

Populations of *M. incognita* were acquired from the Agricultural Research Council, Nelspruit. The identity of *M. incognita* was confirmed using sequence-characterized amplified regions-polymerase chain reaction (SCAR-PCR) (Khosa et al., 2021). *Meloidogyne incognita* populations were multiplied by introducing a sample of confirmed nematodes to susceptible tomato cv. 'Floradade' for two months. Nematode eggs and J2 used were extracted from roots of tomato plants by blending and maceration of plant roots in a 1% NaCl solution (Marais et al., 2017). The aliquot was then sieved through several nested sieves with different apertures: 150, 63, and 25 µm, with inoculum collected from the smallest sieve**.**

Treatments and experimental design

Cassava sprouts of cv. 'Mbonisweni' and 'Mganduzweni' were separately exposed to *M. angolensis* powder levels of 0, 184, 368, 736, 1 472, and 2 944 kg of extract/ha with five replications in a randomised complete block design. Untreated cassava plants were used as negative control while plants treated with Nemacur® (400 g fenamiphos/L) were used as a positive control across all replications. In all replications, a nematode susceptible tomato cv 'Floradade' was included as an indicator of nematode viability.

Data collection

Plant variables: At 72 days after inoculation, the length of sprouts were measured from the basal part to the end of the flag leaf using a 30 cm ruler. Chlorophyll content was measured from the topmost matured leaves using a chlorophyll meter (Spad-502, Minolta, Japan). Sprouts were removed from cutting buds and stem diameter measured 5 cm from the distal end of the cut shoot using a Vernier caliper (GV9370, Grip, Johannesburg). The shoots were then dried in an oven set at 52°C for 72 hours and weighed (Timana et al., 2021).

Nematode variables: Using the blending and maceration method, nematodes were extracted from the total root system per plant (Marais et al., 2017). The sugar-floatation and centrifugation methods were used to extract J2 from soil samples (Marais et al., 2017). Eggs and J2 from cassava roots and soil samples were separately counted from a 1-ml aliquot under a stereomicroscope (Model CX23RTFS2, Olympus Corporation, Tokyo) at X40 magnification. Nematode numbers per 1 ml aliquot were extrapolated to nematodes/total root system, while nematode numbers from the soil were extrapolated to total nematodes per total volume of growing media, all to allow for the determination of final nematode population density (Pf).

Reproductive potential (RP) and reproductive factor (RF) were then computed (Sasser, 1984):

$$RP = \frac{\text{Total nematode population}}{\text{Fresh root mass}}$$

$$RF = \frac{\text{Final population per pot}}{\text{Initial population per pot}}$$

Data analysis

The nematode and plant growth variable data were subjected to analysis of variance (ANOVA) through Statistix10 software. Before ANOVA, Shapiro-Wilk's normality test was used to test for deviation from normality in each standardised residuals variable (Gomez & Gomez, 1983). Inherent variabilities were removed by subjecting data to a Log_{10} (x+1) transformation. Mean separation was achieved using Fisher's least significant difference at 5% probability level.

RESULTS AND DISCUSSIONS

Shapiro-Wilk normality tests indicated that all nematode and plant growth variables were not normally distributed ($P \leq 0.05$), except for chlorophyll content, thus measured variables were transformed accordingly. The cassava root systems were mostly clean of nematode galls, with visible ones being extremely small and underdeveloped.

Plant extract level effects were highly significant ($P \leq 0.01$) for all nematode variables measured including the reproductive factor and potential except for number of eggs in roots ($P \leq 0.05$), while in all plant growth variables there were no statistically significant effects, except for number sprouts, dry shoot mass, plant height and stem diameter (Tables 1 and 2). The reproductive factor were greater than 1 across all the plant extract levels except for at 2 g, which were not different from both the negative and positive control. The reproductive potential was greater than 1 across all plant extract levels except for positive control (Table 1). Across all nematode variables, there were more nematode populations in plants treated with *M. angolensis* except at 6 g, which was not different from both controls. The plant extract generally improved the plant growth variables of cv. 'Mbonisweni' better than 'Mganduzweni' (Table 2).

Table 1. Effect of *Maerua angolensis* application quantity on nematode variables, reproductive factor, potential and stem diameter

Crude extract level	Juveniles in roots	Total nematodes in roots	Stem diameter	Juveniles in soil	Total nematodes in a pot	Reproductive Factor	Reproductive Potential
0	0.9144^{bc}	1.1785^{b}	0.1639^{c}	1.3208^{bcd}	1.9105^{c}	0.3254^{bc}	0.8169^{b}
	(570.00)	(520.00)	(0,4610)	(7600.0)	(8120.0)	(2.7067)	(62.888)
2	1.3857^{ab}	1.4423^{ab}	0.1805^{bc}	0.8486^{cd}	2.0151^{bc}	0.1702^{c}	0.8758^{b}
	(143.23)	(192.06)	(0,5202)	(2508.2)	(2700.3)	(0.9001)	(18.877)
4	1.5415^{ab}	1.6878^{ab}	0.2024^{ab}	2.7947^{ab}	3.7324^{a}	0.5191^{ab}	1.1224^{ab}
	(570.00)	(660.00)	(0,5960)	(9200.0)	(9860.0)	(3.2867)	(88.407)
6	0.5944^{bc}	0.9766^{bc}	0.2198^{a}	2.5112^{abc}	2.8187^{abc}	0.4444^{ab}	0.5926^{bc}
	(136.33)	(177.64)	(0.6584)	(9962.2)	(10140)	(3.3799)	(33.997)
8	2.4700^{a}	2.5357^{a}	0.1894^{abc}	2.0290^{abc}	3.1980^{abc}	0.4678^{ab}	1.7419^{a}
	(755.39)	(876.17)	(0,5538)	(9880.7)	(10757)	(3.5856)	(123.11)

Crude extract level	Juveniles in roots	Total nematodes in roots	Stem diameter	Juveniles in soil	Total nematodes in a pot	Reproductive Factor	Reproductive Potential
10	1.7959[ab] (460.27)	1.8333[ab] (519.21)	0.2115[ab] (0,6320)	3.5130[a] (23731)	3.4559[ab] (24250)	0.8117[a] (8.0833)	1.2377[ab] (62.395)
Nemacur	0.0000[c] (0.0000)	0.0000[c] (0.0000)	0.1870[bc] (0,5440)	0.0000[d] (0.0000)	0.0000[d] (00000)	0.0000[c] (0.0000)	0.0000[c] (0.0000)
F-value	4.31	3.69	2.80	3.56	6.66	4.12	3.52
$LSD_{0.05}$	1.2392	1.3164	0,0351	2.0030	1.6089	0.3951	0.9250
P-value	0.0016**	0.0045**	0.0209*	0.0056**	0.0000**	0.0022**	0.0060**

[x]Column means followed by the same letter are not significantly different at P ≤ 0.05, according to Fisher's least significant difference. Values in brackets are untransformed means. *Significant
(P ≤ 0.05); **Highly Significant (P ≤ 0.01)

Table 2. Effect of *Maerua angolensis* on plant growth variables and nematode eggs in roots

Cultivar	Number of Sprouts [x]	Plant Height	Shoot mass	Dry Shoot mass	Eggs in Roots
Mbonisweni	0,4758[a] (2,1143)	1,8498[a] (73.691)	1,3504[a] (26,158)	0,8775[a] (7,6054)	0,3828[b] (34.286)
Mganduzweni	0,3502[b] (1,2867)	1,6932[b] (50.169)	0,9525[b] (9,792)	0,5771[b] (3,2423)	1,0371[a] (85.675)
F-value	20.39	19.32	25.26	26.62	7.16
$LSD_{0.05}$	0,0560	0,0717	0,1593	0,1172	0,1094
P-value	0.0000**	0.0001**	0.0000**	0.0000**	0.0103*

[x] Column means followed by the same letter are not significantly different at P ≤ 0.05, according to Fisher's least significant difference. Values in brackets are untransformed means. *Significant (P ≤ 0.05); **Highly Significant (P ≤ 0.01).

Figure 1. Cassava cv. 'Mbonisweni' inoculated with *Meloidogyne incognita* and treated with *Maerua angolensis*

Even though there was evidence of nematode viability on indicator tomato plants, the current study was not conclusive. Higher nematode levels were observed in soils with plants exposed to plant extracts compared to the negative controls and the root system of cassava. The application of *M. angolensis* plant extracts seem to increase *M. incognita* populations in pots and reproductive factor of the nematodes. The observations contradict reports made on tomato plants by Khosa et al. (2021; 2020a; 2020b; 2013). Khosa et al. (2020a; 2020b) reported that *M. incognita* eggs and second-stage juveniles were highly sensitive to *M. angolensis* under *in vitro* and greenhouse conditions. Khosa et al. (2020b) using a CARD Model demonstrated that *M. angolensis* has very high potency on hatching and mortality rate of *M. incognita*. Stem extracts of *M. angolensis* have also been reported to control *Haemonchus contortus* in sheep (Fouche et al., 2016). These variations could be because the powdered extracts largely depend on water to dissolve, it is possible that the outcomes could be different if the plant extracts used are applied in a liquid form.

Secondary metabolites in plants have been used as defensive strategies against insect pests and or pathogenic organisms (Sithole et al., 2021). Because of the growing interest to find an environment-friendly way of managing root-knot nematodes, different plant species have been reported to suppress nematode populations (Asif., 2017; Ntalli & Caboni, 2012). Some of the most investigated plants comprise elderberry (*Sambucus nigra* L.) (Akyazi, 2014) and garlic (*Allium sativam* L.), mugwort (*Artemisia vulgaris* L.) (Khosa et al., 2021).

Reproductive factor (RF) is used to describe host status, which is a measure of the nematode's ability to reproduce in a host. A reproductive factor greater than one, indicates that nematode reproduced in the plant whereas the RF less than one, indicates the inability of the nematode to reproduce in a plant. Sasser et al. (1984) included the galling index together with the RF to describe the plant status, when the gall index is less than two and reproductive factor is less than one, the plant is said to be resistant. A plant is regarded as tolerant when the gall index is less than two and reproductive factor greater than one, whereas, when the gall index is greater than two and nematodes managed to reproduce, the plant is considered to be susceptible (Adegbite, 2017). In this study, RFs were greater than one except at 2 g, and there were few small galls on the plant roots and tubers, making the gall index in this case, less than two.

With the limited information gathered on the response of tolerant plants to plant extract used in nematode management, the results of the current study become difficult to explain. Dube (2016) observed a stimulative effect of cucurbitacin and cucurbitacin-containing plant extracts on *M. incognita* J2 hatch and mobility when exposed to low concentrations under *in vitro* conditions. The behaviour of plant-parasitic nematodes is continuously influenced by chemical cues in their environments, these chemicals can repel, attract or kill the nematode (McSorley, 2003). A larger body of evidence on the stimulatory effects of plant extracts has been associated mainly with beneficial nematodes, such as, *Sternernema* species (Madaure, Mashela & De Waele, 2017) and other plant beneficial organisms such as nitrogen-fixing bacteria (Mashela & Pofu, 2012). The mechanism by which plant extracts affect beneficial organisms has taken a variety of forms, from changing the soil properties to providing food (Widmer, Mitkowski & Abawi, 2002).

The effect on the cassava plant cannot be left out in the outcome of this study, especially considering that the negative control plants responded the same way as the plants exposed to extracts and positive control on most variables measured. Similar trends as in the study by Timana et al. (2021) were observed in the current one, where there was an increase of nematodes in soil than in roots and reproductive factor response, support this. The cassava plants' ability to tolerate the nematode could have had a much greater effect on nematode behaviour than plant extracts and knowing the chemistry of the plant extracts could help in better understanding this outcome.

A more comprehensive study needs to be conducted to substantiate the interactions between, *M. incognita*, cassava plants, and *Maerua angolensis* plant extracts. This work could include the mode of interaction of the three, the effect of space, the active ingredients of the plant extract and time on the three above-mentioned factors.

CONCLUSIONS

Based on RF values obtained in this study and the gall index of less than 2, both cultivars are tolerant to *M. incognita*. The cassava plants' ability to tolerate the nematode could have had a much greater effect on nematode behaviour than plant extracts and knowing the chemistry of the plant extracts could help in better understanding this outcome.

The study did not provide a conclusive relationship between the cassava plants, *M. angolensis,* and *M. incognita.* The use of *M. angolensis* plant extract did not bring any added benefit to the crop or suppress nematode populations in cassava cv. ‘Mbonisweni’ and cv. ‘Mganduzweni’.

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THE PERFORMANCE OF THE PERSIMMON GROWN IN THE SOUTHERN OF ROMANIA

Irina TITIRICĂ[1], Florentina NETCU[1], Cristina BÎRSOGHE[1], Ioana ROMAN[2]

[1]Research and Development Station for Plant Culture on Sandy Soil Dăbuleni, 217 Petre Banita Street, Dăbuleni, Romania
[2]Department of Transversal Competences, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: titiricairina@gmail.com

Abstract

In Romania until now, are no commercial persimmon (Diospyros kaki Thunb.) orchards. But, the interest for the fruits of this species is increse. Solitary trees of Diospyros virginiana being found in parks and private gardens it have fruits are very astringent, without interest for consuummers, but they offer a very atractive wiew in lands to the starting of the winter season. In the South of Romania, during the winter, are occasional registered temperatures of -20-25°C which cane affects the varieties from Diospyros kaki. For this reason, in 2019 year, we established an experimental plot with two cultivars Rosseyanka and Jiro, to study the persimmon culture suitability in Romania. The study carried out so far, shows that the Rosseyanka cultivar, reached a high fruits yield of 11 kg/tree, in the fourth year after planting. From the point of view of fruit quality, the Jiro variety recorded a value of fruit weight of 162.2g/fruit and 85.36 mg% vitamin C fruits contents.

***Key words**: yield, fruit quality, biometrics indices.*

INTRODUCTION

Currently in the world there is a tendency to change eating habits. On the one hand, the emphasis is on maintaining and providing traditional, local foods, on the other hand, new resources are being sought to change eating habits. In this context, the consumption of the fresh and processed fruits is also included. Generaly, in the fresh fruit market, in search of an elixir for health, the consumers are looking for fruits whose species are less cultivated, but are traditionally consumed species in Asian countries, countries considered to have the population with the longest and healty live. Thus, the fruits of the *Diospyros kaki* Thumb species entered the market, numerous studies show that fresh kaki fruits have antitumor effects, prevent dyslipidemia, have anti-hyper-cholesterolemic effects, have antioxidant and antidiabetic properties. The antioxidant effects are due to the wealth of vitamins, phenolic compounds and carotenoids (Direito et al., 2021)

Diospyros kaki Thunb., is a species native to China. It has been cultivated here for over 2000 years, over extensive areas from altitudes of 20 m to altitudes of 600-800 m (George et al., 1990). In Europe this species has been known during the age of Plinio, Roman works show that some persimmon trees were cultivated in Rome since then. Spaniards during the Colony carried out a quite important introduction of plants from Far Eastern countries (Belini and Giordani, 2003).

Today, kaki has grown in popularity outside of its traditional production region, becoming a thriving crop in Brazil and some Mediterranean countries such as Italy Spain and Grace (Guan et al., 2020).

Currently, the largest collection of Diospyros species genotypes is in Italy (125 genotypes) (Lim et al., 2012). The germplasm collections from the larges producing countries as well as the local biotypes are a support source for the genetic diversity, to the breeding activity in order to create genotypes with increased adaptability to the increasingly evident conditions of climate change (Samarina et al., 2021).

In Europe the cultivation of persimmon is limited to the proximity to the Mediterranean Sea (Direito et al., 2021). In France, the first persimmon plantations were established in 1970, In Portugal in the Algarve region, is a area with orchards dedicated to persimmon, altought the persimon trees are scattered throughout the central and northern regions of the country. In the countries on the Mediterranean coast as Italy, Spain, Greece, Turkey the persimons orchards are in extension, also (Yesiloglu et al., 2022). Now, the world production of persimmon is around five million tons (Direito et al., 2021).

In Romania, due to its temperature requirements (at -18°C the anual shoots are affected in winter), this species *Diospyros kaki* has not been yet cultivated in commercially orchards, although genotypes from *Diospyros virginiana* L. give beautify the parks in our country. The genotypes from *Diospyros virginiana* L., beiing from North America, their fruits have a attractive wiev, like the Chrismas ornaments in the winter, but do not lose their astringency at full maturity, which makes them unattractive for fresh consumption. But this species is an important genetic resource, usually used in crosses with *D. kaki* in breeding programs for frost resistance and as rootstock also in propagation activity.

In our country, in Romania, studies on the behavior of 16 biotypes of *Diospyros virginiana* and *Diospyros lotus* were initiated by Dr. Mladin Gheorghe, at the Research Institute for Fruit Growing Pitesti-Mărăcineni, but they were not extended into orchards.

Thus, currently there are no commercial persimmon plantations in the country, but this fruit is sold by the piece in large stores.

The purpose of this paper is to highlight the growth and fruiting performance of 2 genotypes: Rosseyanka, an interspecific hybrid *Diospyros virginiana* x *kaki*, and the genotype Jiro from the species *Dyospiros kaki*, in the pedoclimatic conditions of southwest Romania with a view to expanding into commercial crops.

MATERIALS AND METHODS

In the period 2019-2022, within the Research and Development Station for Plant Culture on Sandy Soil Dăbuleni, Romania, two genotypes *Rosseyanka* (Photo 1) and *Jiro* (Photo 2) were studied, planted at a distance of 5 x 5 m, on raised beds, mulched with agrotextile and irrigated with a drip irrigation system under mulches.The location of the experimental plot was 43 80 63 N and 24 95 96 East on a sandy soil poorly in nitrogen (0.02%), phosphorus (25 ppm), and potassium (36 ppm) with very low organic carbon content (0.07%) all those are characteristic of sandy soils, and the soil reaction was neutral (pH 6.36).

For evaluate the growth capacity, the following determinations were made: the diameter of the trunk (cm) was measured at the beginning of the vegetation period 10 cm from the grafting point, marking the reading point with paint; the dynamics of shoot growth (cm) was recorded by monthly measurement of annual shoots; the surface of the tree trunk cross sectional (TCSA) was calculated according to the formula: πr2 considering the circular trunk (Toplu C. et al., 2009) and the canopy volume (CV) was measured before pruning, calculated according to the formula CV= 4/3πab2, where a is the axis length/2, and b is the minor axis length/2 (Toplu C. et al., 2009).

To evaluate the fruits quality, samples of 30 fruits was evaluated annually in three repetitions for determining the average weight of the fruits (g); as well the fruits diameter (mm); the fruits height (mm) by measuring every fruits with a digital caliper; the fruits color indices were measured using the Hunter system, where L*(brightness); a*(redness +, greenness -); b*(yellowness + or blueness-) with the PCE-XXM20 colorimeter. For L*a*b* values hue angle was calculated as $h^o = tan^{-}(b^*/a^*)$ (Abbott, 1999) and Chroma index as $C = (a^{*2}+b^{*2})^{1/2}$ (Lopez & Gomez, 2004). The color values and firmness for each fruit were computed as means of two measurements taken from both sides at the ecuatorial region of the fruit. The fruits colour and the firmness were measured both at the time of harvest and 28 days after harvest when the fruits became soft good for consumption, the firmness of the fruits were determined with the PCE-FM 200N penetrometer. The yield (Y) per hectare (ha) was estimated by calculating as Y = average yield per tree x no. trees/ha (400 trees/ha). The biochemistry of the fruit at harvest maturity were made as follows: soluble dry matter (% Brix) was made with the Atago apparatus; the vitamin C content (mg%) was determined by the iodometric method (Croitoru 2021); the glucides content (%) was made by the Fehling Soxhlet method, and titratable acidity (%) was determined by the titrimetric method.

Photo 1. The *Rosseyanka* cultivar

Photo 2. The *Jiro* cultivar

The obtained results are presented in tables and figures, and the bars in each column of the figures represent the standard deviation.

RESULTS AND DISCUSSIONS

The illustration of the trees trunk diameter evolution in table 1, shows that the Jiro cultivar on the study period had registered the highest values versus the *Rosseyanka* cultivar. So, since the first year (2020) to the planting, has recorded an increase of 12.3 cm, and in the second year after planting (2021 year), the recorded values were 14.9 cm higher than the average values of the trunk diameter recorded by the *Rosseyanka* cultivar. Analyzing the increase of trunk registered by each cultivar, from one year to another, we find that the *Rosseyanka* cultivar registered an annual increase in trunk diameter of 4.60 cm in 2021 year compared to 2020 year and 6.90 cm in 2022 year compared to from the previous year (Table 1). The recorded trunk diameter data were compared with those recorded by Omarov et al. (2022) over a 5-year average, at a planting distance of 6 x 3, and show that the two cultivars developed normally between our data and those recorded in Russia being a difference of only 1.27 cm. Regarding the tree trunk cross sectional (TCSA), an increase from one year to the next is found between 14.45 and 21.66 cm^2, in the case of the *Rosseyanka* cultivar and between 9.11 and 22.6 cm^2, in the case of the *Jiro* cultivar. Comparing the data recorded under the conditions in southern Romania with those recorded by Toplu et al. (2009), in Turkey shows that the *Jiro* cultivar registers under the conditions in Romania an increase of 20.63 cm^2, in the fourth year after planting versus the data registered in Turcia by the same cultivar.

The average fruit production per tree was 8.5 kg in the case of the *Rosseyanka* cultivar, respectively 0.121 kg/cm^2 trunk section and only 0.048 kg/cm^2 in the case of the *Jiro* cultivar. The data on fruit production per cm^2 of the trunk section were compared in the case of the *Jiro* cultivar with those recorded by Toplu & all. (2009), in the 7th year (1.4 kg/cm^2) from planting and show that they are obviously higher with 1.1kg/cm^2 trunk section. The *Rosseyanka* variety recorded a canopy volume of 1.15 cm^3, an average value lower by 1.0 m^3 compared to the canopy volume recorded by the *Jiro* cultivar. In terms of fruit production per ha, the most productive cultivar that stood out was the *Rosseyanka* cultivar registering a higher production by 1.4 t/ha versus to the *Jiro* cultivar (Table 1).

Tabel 1. The evolution of trunk diameter in the studied period and the yield

Cultivar	The trunk diameter			TCSA (cm²)			CV (m³)	Yield / tree (kg)	Yield/ cm² of TCSA (kg/cm²)	Yield/ha (t/ha)
	2020	2021	2022	2020	2021	2022	2022	2022	2022	2022
Rosseyanka	10.7	15.3	22.2	33.59	48.04	69.70	1.15	8.5	0.121	3.4
Jiro	23.0	30.2	33.1	72.22	94.82	103.93	2.15	5.0	0.048	2.0
St. Dev.	6.15	7.45	5.45	19.31	23.39	17.11	0.5	1.75	0.036	0.7

The analysis of the growth dynamics of the shoots shows that until September 10, the vegetative growth recorded a monthly increase between 15 and 20 cm for both genotypes, and the vegetative growth stopped only in September. On average over the entire vegetation period, the *Rosseyanka* cultivar recorded an average shoot length of 59.59 cm, and the *Jiro* cultivar recorded an average length of 53.04 cm (Figure 1). The data recorded, in the specific conditions of the sandy soils in the southwest of Romania, were compared with those in the subtropical region of Russia, and it was found that the average length of the annual shoots in the conditions of Romania are with 10.20 cm higher than those recorded by Omarov in 2017 in Russia.

The analysis of the fruits biometric characteristics showed that the average values of the calix compared to the fruit diameter, in the case of the *Rosseyanka* cultivar, cover 45.3% of the fruit diameter, and in the case of the *Jiro* variety, the calix covers only 37.8% of the fruit diameter (Table 2). For four of the three biometric indicators studied, the *Jiro* cultivar recorded the highest values. The comparison of fruit firmness data shows that both at picking and at maturity *Rosseyanka* cultivar recorded values 0.62 (kgf/cm²) higher than the *Jiro* cultivar, and at maturity the difference between the two varieties was only 0.03 kgf/cm² (Table 2). Regarding the fruit color measured in the Hunter system, in the case of fruits brightness (L*), the data show that at the time of harvest the *Rosseyanka* cultivar recorded the higher value by 4.9% versus the *Jiro* cultivar.

The recorded data were compared with those of Senica et al. (2016) show that under the Romanian conditions the recorded values are higher by 10.9% in the case of the *Jiro* cultivar and by 13.9% in the case of the *Rosseyanka* cultivar. But these differences in brightness can also be influenced by the device with which the determinations were made, these being different. The comparison of the brightness data at the time of harvest and at maturity shows that in the case of the *Jiro* cultivar the values did not change, on the other hand, in the case of the *Rosseyanka* cultivar the values at the consumption maturity were 2.8 units lower than those recorded at harvest (Figure 2). The calculation of the color index shows that to both cultivarss the values of both index (h and C) increase at consumpion maturity by 13.54 and by 28.3 in the case of the hue angle, and in the case of the Chroma index the values increased by 3.15 and respectively 27.75 (Figure 2).

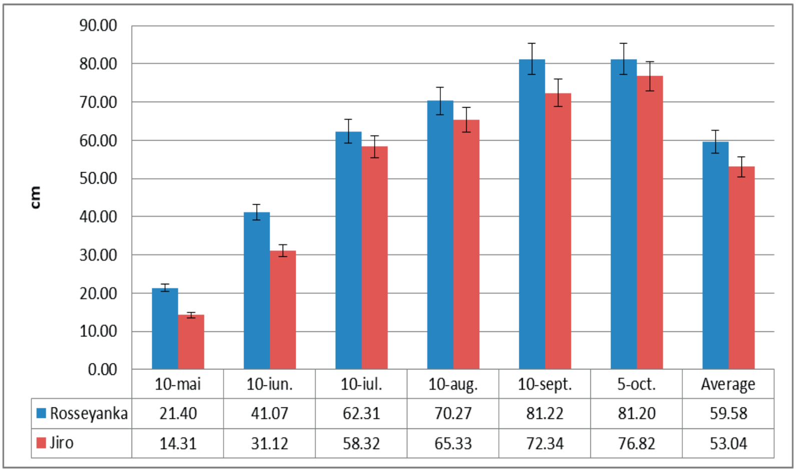

Figure 1. The dynamics of shoot growth in the studied period

Table 2. The biometric fruits characteristics

Variety	Average fruit weight (g/fruit)	Fruit firmness (Kgf/cm^2)		The fruit height (mm)	The fruit diameter (mm)	The calix (mm)
		at picking	at consumption maturity			
Rosseyanka	76.9	1.61	0.18	40.59	50.803	27.77
Jiro	182.3	0.99	0.21	49.07	69.443	43.14
STDEV	74.53	0.44	0.02	6.00	13.18	10.87

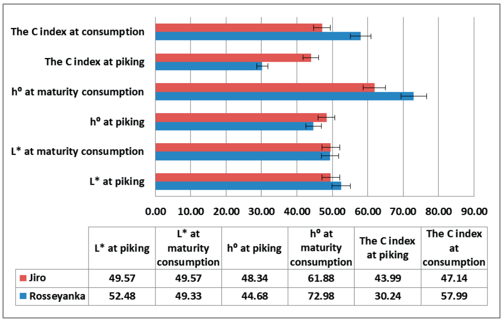

Figure 2. The biochemical characteristics of the fruits

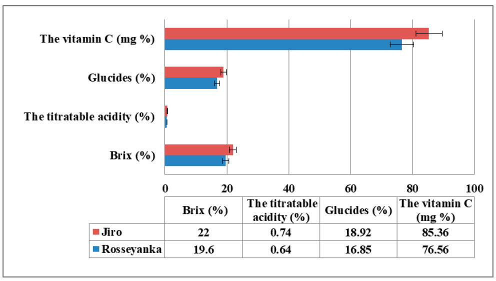

Figure 3. The fruits colour index

The evaluation of the biochemical characteristics of the fruits highlighted the *Jiro* cultivar that recorded the highest values for all 4 biochemical properties of the fruits (Figure 3).

CONCLUSIONS

The vegetative growth of the trees as well as the production recorded in the 4th year after planting are consistent with the specialized literature.

From the productive point of view, the Rosseyanka variety stood out, and from the fruit quality point of view, the Jiro variety stood out by the appearance of the fruits (average fruit weight over 150g/fruit) as well as by the analyzed biochemical properties.

As a result of the study, it was found that both persimmon varieties found favorable conditions for growth and fruiting in the specific conditions of the sandy soils at SCDCPN Dabuleni.

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STUDY OF THE NEW CLONAL CHERRY ROOTSTOCK HYBRIDS 20-192 AND 20-181 IN NURSERY

Dimitar VASILEV[1], Svetoslav MALCHEV[2], Lilyana NACHEVA[2]

[1]Konstantin Preslavsky University of Shumen, 115 Universitetska Street, Shumen, 9700, Bulgaria
[2]Fruit Growing Institute - Plovdiv, Agricultural Academy, 12 Ostromila Street, 4004, Plovdiv, Bulgaria

Corresponding author email: d.v.dimitrov@shu.bg

Abstract

Studies have been conducted in the fruit tree nursery of Agricultural Experiment Station - Khan Krum between 2013 and 2016. Research subjects were the selected cherry rootstock hybrids 20-181 and 20-192 as clonal rootstocks grafted with cultivars Kossara, Trakiiska hrushtyalka and Van. Prunus mahaleb seedlings were used as a reference. The aim of the study was to investigate the growth characteristics, quality of planting material, and compatibility of the rootstocks with commercial cultivars. The average success rate of grafting reported in the autumn of the analyzed cultivar-rootstock combinations varied as follows: P. mahaleb (89-92%), hybrid 20-192 (85-89%), and hybrid 20-181 (81-86%). The clonal rootstock 20-192 induces relatively weaker growth than the Mahaleb. The weakest growth characterizes hybrid 20-181. Both tested rootstock hybrids, obtained by the crossing of 'Polevka'×'Compact Van', produce planting material with standard trunk diameter and tree height.

Key words: *sweet cherry, Prunus mahaleb, rootstocks, cultivars, hybrids.*

INTRODUCTION

The rootstock plays an increasingly important role in the application of new technologies and the development of modern fruit growing.
In the last 20 years, both new technologies and many newly selected rootstocks have entered cherry production (Lichev et al., 2020). The selection of new cherry rootstocks is related to the creation of intensive plantings, the introduction of new training systems, and the production of higher yield and better quality (Lang, 2000; Hrotko et al., 2007; Hrotko, 2009; Hrotko, 2010; Lichev, 2015; Pal et al., 2017; Kaplan et al., 2018).
The search for less vigorous cherry rootstocks began relatively late in the 1960s after it had already been demonstrated in pome fruit species the advantage of week-growing trees and the greater density of plantations (Webster, 1989). As a result of targeted selection activity over the last 20 years of the past century, a relatively large number of rootstocks with moderate and even weak growth have been created throughout the world (Trefois, 1989; Schimmelpfeng, 1993; Sansavini & Lugli, 1996; Franken-Bembenek & Ystaas, 1998; Quero-García et al., 2017). As a result of targeted selection activity, a relatively large number of rootstocks with moderate and even low growth were created in the last 20 years of the past century (Franken-Bembenek., 1996; Lichev, 2015; Quero-García et al., 2017; Lichev et al., 2020).
In Bulgaria, the fastest spreading in practice are the rootstocks from the series 'GiSelA®', mainly 'GiSelA 5' and 'GiSelA® 6'.
According to Manolova & Kolev (2012), the 'GiSela 5' rootstock is the most economically efficient in establishing new orchards but requires a high level of agrotechnical orchard management and if it is not provided, good economic results cannot be expected. And for container growing Akova (2022) recommends GiSela 6 rootstock to be planted in containers with a volume that does not exceed 5 l.
The analysis of the state of the problem reveals that data on the Bulgarian selection of cherry rootstocks with weak vigor is very scarce. The aim of the present study is to test cherry rootstocks selected in Bulgaria with weak vigor and to compare them with the rootstock most used in Bulgaria rootstock for the production of planting material.

MATERIALS AND METHODS

The trial was set in the fruit tree nursery of the Experimental Agriculture Station - Khan Krum. The rootstocks used in the evaluation were the new clonal rootstock hybrids from the Fruit Growing Institute - Plovdiv - hybrids 20-181 and 20-192 with *Prunus mahaleb* seedlings as reference.

T-budding was performed at the beginning of August in the first year, and each of the three rootstocks was budded with cultivars 'Van', 'Kossara', and 'Trakiiska hrushtyalka'.

The two hybrids 20-181 and 20-192, being tested as rootstocks for sweet cherry cultivars, are obtained from the parental combination ('Polevka'×'Compact Van') producing a population of 31 hybrids, out of them the two were selected for their weak growth and drought and pest resistance. The cultivars 'Kossara' and 'Trakiiska hrushtyalka' were obtained also as a result of the breeding program of the Fruit Growing Institute - Plovdiv - 'Kossara' from crossing 'Ranna cherna' × 'Bigarreau Burlat', and 'Trakiiska hrushtyalka' from open pollination of 'Van' (Zhivondov, 2012; Malchev, 2016).

The observations were carried out in the period between 2013 and 2016 in first- and second-year nursery. The planting distances in the nursery were 80/12 cm.

During the vegetation, growth dynamics were recorded every 10 days/from 20.05 to 18.9/. The trunk thickness was measured at 15 cm from the soil surface with a micrometer. The nursery is under irrigated conditions and standard cultivation techniques. The statistical analysis of the data was carried out according to the Dunkan MRT (De Mendiburu, 2021).

On the basis of the presented Table 1 meteorological data for the four-year period, we can draw the conclusion that precipitation during the growing seasons of the trial is unevenly distributed, and average monthly temperatures have large amplitudes in individual years.

Table 1. Mean monthly air temperature ^{0}C and precipitations totals in 2013-2016 relative to the long-term means

	Temperature (^{0}C)					Precipitation (mm)				
Month	2013	2014	2015	2016	Long-term mean	2013	2014	2015	2016	Long-term mean
January	0.8	2.8	2.0	2.3	4.0	32.5	50.3	38.0	80.0	35.0
February	3.1	4.0	3.2	3.6	2.5	57.9	40.3	61.0	21.4	28.0
March	5.4	9.2	5.7	9.2	7.1	42.0	51.2	61.0	60.8	31.0
April	14.8	12.8	11.7	14.3	10.9	37.4	53.1	55.0	41.0	41.0
May	18.3	15.8	19.5	14.8	16.7	81.5	158.2	26.0	75.5	64.0
June	23.9	20.3	21.1	21.5	21.6	34.0	169.5	39.0	44.0	75.0
July	22.6	24.5	25.8	23.0	23.4	135.0	31.0	27.0	70.0	60.0
August	24.4	24.8	25.8	22.2	22.3	46.9	84.1	68.0	63.0	53.5
September	19.2	19.3	19.2	18.7	17.9	43.2	142.5	89.5	0	3.0
Oktober	14.3	12.7	12.8	10.1	12.9	56.4	75.2	68.0	43.0	78.5
November	8.8	11.4	7.3	6.3	6.8	57.7	89.2	45.0	67.0	51.0
December	1.2	3.4	4.6	-0.4	1.8	6.8	133.9	38.0	16.3	57.0

RESULTS AND DISCUSSIONS

Table 2 presents data on the average success rate for the autumn and spring T-budding of the Cultivars 'Kossara', 'Trakiiska hrushtyalka' and 'Van' during the period 2013-2016. Autumn bud success rate ranged from 90.0 to 98.0% for the different scion/rootstock combinations.

The values obtained during spring reporting are relatively lower than those obtained in autumn. The highest percentage of successful budding in spring was found in the three varieties bred on *P. mahaleb* seedlings (89.6-92%).

Table 2. Percentage of T-budding success rate (average for the period 2013-2016)

Cultivar/Rootstock	Autumn Reporting, %	Spring Reporting, %
Kossara / *P. mahaleb*	98.0 a	89.6 ab
Kossara / hy. 20-192	94.0 cd	85.1 bcd
Kossara / hy. 20-181	90.0 e	81.0 d
Trakiiska hrushtyalka/*P. mahaleb*	97.0 ab	92.0 a
Trakiiska hrushtyalka /hy. 20-192	92.0 de	87.4 abc
Trakiiska hrushtyalka hy. 20-181	91.5 de	83.0 cd
Van / *P. mahaleb*	93.6 cd	90.2 ab
Van / hy. 20-192	95.2 bc	89.3 ab
Van / hy. 20-181	90.1 e	86.1 abcd

**different letters in the same column mean a significant difference at P = 0.05

The cultivars 'Van', 'Kossara' and 'Trakiiska hrushtyalka' grafted on the rootstock hybrid 20-192 are characterized by a relatively high success rate (85.1-89.3%). Successful budding using the clonal rootstock hybrid 20-181 was relatively low (81-86.1%).

The resulting rate is good, but slightly lower than the results shown on the *P. mahaleb* seedlings and hybrid 20-192. This can be explained by the weaker growth of the rootstocks and the slower reaching of the thickness necessary for grafting a dormant bud.

The data presented in Figure 1 show the growth dynamics of the cultivars 'Van', 'Kossara' and 'Trakiiska hrushtyalka' budded on the clonal hy. 20-181, hy. 20-192 and *P. mahale*b seedlings.

'Kossara' cultivar scions on the Mahaleb rootstock are comparatively the strongest growing. The first peak of height growth is observed from 30 May to 19-July, and the second from 19 July until 08 September.

'Kossara' plants on rootstock hy. 20-192 have comparatively lower values than those grown on Mahaleb, characterized by a fast initial growth rate in the period from 29 June
until 19 July (30.0-63.5 cm).

The rootstock hybrid 20-181 induces the scion ('Kossara') a relatively slow growth rate in height. The cultivar grafted on it grows most intensively from 19-July to 18 August (35.0-80.7 cm). In the period from 18 August to 08 September, the oculants reach a height of 80.7 to 97.1 cm. The last two measurements are characterized by stunted and very weak growth.

Strong growth is observed in cultivar 'Trakiiska hrushtyalka' budded on Mahaleb rootstock. Compared to the 'Kossara' cultivar, 'Trakiiska hrushtyalka' is distinguished by a stronger growth rate that is maintained from the beginning to the end of the vegetation (08-September). In the period from 09 June to 09 July, the scions grow to a height of 25.1 to 78.5 cm. On 19 July, plant height of 100.4 cm was recorded, which is a good indicator of the quality of the planting material. The second peak of growth is observed from 19 July to 08 September.

Budded on a rootstock 20-192 hybrid, the cultivar 'Trakiiska hrushtyalka' has relatively smaller trees than those budded on Mahaleb. The selected rootstock hybrid 20-192 induces 'Trakiiska hrushtyalka' moderate to vigorous growth. A rapid rate of growth of the scions was observed in the period from 09 June to 28 August, with the plants reaching a height of 153.2 cm.

Clonal rootstock hybrid 20-181 induces relatively weak growth to 'Trakiiska hrushtyalka' scions. When using 20-181 as a rootstock, standard trees with a height of 103.5 cm can be obtained.

The standard cultivar 'Van' is still widespread in production in Bulgaria. When using a *P. mahaleb* seedlings as rootstocks, a rapid rate of growth in height is reported in the period from 16 June to 08 September (30.3-156.3 cm). The resulting planting material is characterized as strong growing compared to the other rootstocks 20-192 and 20-181. When using the selected hybrid 20-192, the most intensive increase in height was observed in the period from 09-June to 08 September (28.1-140.3 cm). As with other scion/rootstock combinations, rootstock 20-192 induces 'Van' scions moderate to vigorous growth. Examining the data obtained from the cultivar 'Van' budded on rootstock 20-181, it is evident that the scion is characterized by moderate growth without significant growth from 20 May to 29 July. A peak in height growth is observed from 18 August to 18 September (83.3-110.2 cm).

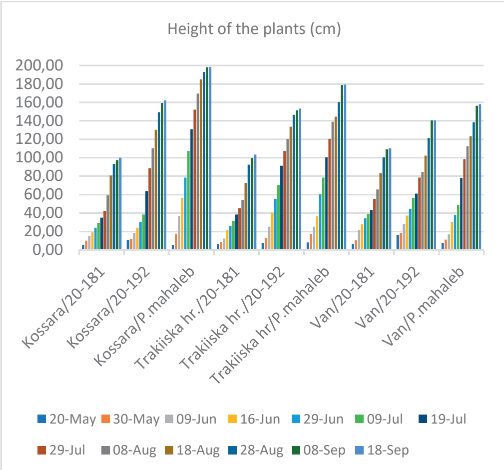

Figure 1. Growth dynamics of the cultivars 'Van', 'Kossara' and 'Trakiiska hrushtyalka' budded on the clonal hy. 20-181, hy. 20-192 and *P. mahaleb* seedlings

Figure 2 presents the data on scion thickness growth of the studied scion/rootstock combinations.

The data presented reveals that the scion/rootstock combination Kossara/*P. mahaleb* is distinguished by the highest values compared to the others. On 09 July, it reached a scion thickness of 10.6 mm.

'Kossara' budded on hybrid 20-192 demonstrated moderate growth in the period from 30 May to 19 July when scion thickness of 1.2 to 6.4 mm was reported. The period from 19 July to 18 September is characterized by a rapid growth rate, increasing the thickness from 6.4 to 16.2 mm.

The same trend is observed in cultivar 'Trakiiska hrushtyalka', as the Mahaleb rootstock induces strong growth, hybrid 20-192 is characterized by strong to moderate scion thickness, and the rootstock hybrid 20-181 induces the scion with weak growth.

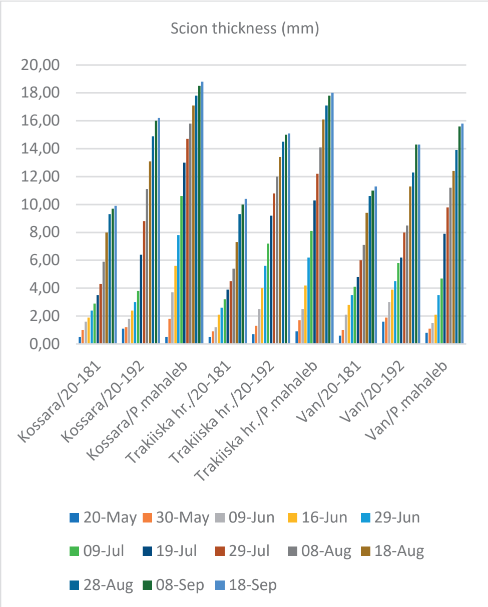

Figure 2. Scion thickness growth o of the cultivars 'Van', 'Kossara' and 'Trakiiska hrushtyalka' budded on the clonal hy. 20-181, hy. 20-192 and *P. mahaleb* seedlings

The reference cultivar 'Van' budded on *P. mahaleb* seedlings and rootstocks from the newly selected hybrid 20-192 has weak vigour during the period from 20 May to 19 July, after which intense scion thickening begins from 19 July to 18 September. The scion/rootstock combination Van/20-192 reached a thickness greater than 10 mm on 18-August and the combination Van/*P. mahaleb* reached the same thickness earlier on 08 August. The last two measurements are characterized by decrease of the rate of the scion thickening. Noteworthy is the rate of thickening of the scion of 'Van' cultivar on rootstock 20-181. In the period from 30 May to 08 August, it is moderate and no large amplitudes are observed. A rapid rate of scion thickening was observed from 08 August to 08 September when values increased from 7.1 to 11.0 mm.

Table 3. Sizes of the obtained trees in the second-year nursery (average for the period 2013-2016)

Cultivar/rootstock	Van	Trakiiska hrushtyalka	Kossara
Height [cm]			
P. mahaleb	158.0 b	179.3 a	198.4 a
Hy. 20-192	140.3 b	153.2 b	162.3 ab
Hy. 20-181	110.2 c	103.5 c	100.0 c
Thickness [mm]			
P. mahaleb	15.8 ab	18.0 a	18.8 a
Hy. 20-192	14.3 b	15.1 b	16.2 ab
Hy. 20-181	11.3 c	10.4 c	9.9 c

**different letters in the same column mean a significant difference at P = 0.05

Table 3 presents the height and thickness data of the planting material (fruit trees) obtained in the second-year nursery.

From the obtained results it is evident that the cultivars 'Van', 'Kossara' and 'Trakiiska hrushtyalka' produce the tallest trees when budded on a *P. mahaleb* seedlings (158.0- 198.4 cm). The same tendency is observed in the thickness of the scion (15.8-18.8 mm). The values for cultivars 'Van', 'Kossara' and 'Trakiiska hrushtyalka' on the newly selected rootstock hybrid 20-192 were relatively lower, respectively 140.3-162.3 cm for the height and 14.3-16.2 mm for the thickness. Rootstock hybrid 20-181 induces scions with the weakest growth and scion thickening and is of interest both in the production of planting material and in the establishment of intensive cherry orchards.

CONCLUSIONS

The highest success rate in spring was reported in the three cultivars budded on *P. mahaleb* seedlings (89.6-92%). The cultivars 'Van', 'Kossara', and 'Trakiiska hrushtyalka' budded on the rootstock hybrid 20-192 are characterized by a relatively high success rate (85.1-89.3 %). The success rate when using rootstock hybrid 20-181 was the lowest (81-86.1 %).

A trend is observed in the cultivars 'Van', 'Kossara', and 'Trakiiska hrushtyalka' budded on *P. mahaleb* seedlings to have strong growth and thickening of the scion, while hybrid 20-192 is characterized by strong to moderate growth and scion thickening, whereas hybrid 20-181 induces the scions the weakest growth.

The resulting fruit trees obtained when using the three rootstocks in combination with the tested cultivars meet the requirements for standard planting material in the country.

The rootstocks hybrids 20-192 and 20-181 deserve attention and would find their place in the establishment of intensive cherry plantations.

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IMPACT OF FERMENTATION TEMPERATURE AND DURATION ON ANTHOCYANIN CONCENTRATION AND ON QUALITY OF CABERNET SAUVIGNON WINE

Géza BALLA, Tekla Amália LUNKA*, József JOÓ, Csaba MOLDOVÁN, Zsolt SZEKELY-VARGA, Artúr Botond CSORBA, Endre KENTELKY*

Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Department of Horticulture, Calea Sighișoarei 2, 540485 Târgu Mureș, Romania

Corresponding authors email: tekla.lunka@gmail.com and kentelky@ms.sapientia.ro

Abstract

Romania has a good geographical location and favourable climate and soil properties for grape growing. The main reason for this is the high anthocyanin content in red wine, which acts as an antioxidant. The aim of the experiment was to examine the effect of changes in fermentation temperature and duration of fermentation on the anthocyanin content and quality of the resulting varietal wines. Also, to improve the quality during the fermentation process of one of the main red wine varieties of the Miniș wine region, Cabernet Sauvignon. The laboratory tests showed that none of the parameters were affected by the fermentation temperature or the duration of the fermentation. In terms of anthocyanin content better results were obtained with longer fermentation period and higher fermentation temperature. Cabernet Sauvignon had an anthocyanin content of 485 mg/L after 15 days of fermentation at 30°C. During the sensory evaluation it can be stated that wines fermented for a longer period of time and at higher temperature proved to get higher score.

***Key words**: Cabernet Sauvignon, fermentation, Miniș, wine.*

INTRODUCTION

Winegrowing and winemaking have a very long history in certain areas of the world where the climatic conditions are favourable. The wine was the jewel for Egyptian, Greek and even Roman cuisine. For a very long time, wine was even more popular for noblemen than water because it was cleaner than the water that was accessible (Kádár, 1973).
Red wine is not just an article of pleasure, it also has a medicinal effect. At the end of the 20th century, the millennia-old fact that wine has an antibacterial effect was scientifically proven. Pasteur's opinion was that wine is the most hygienic and healthiest drink (Stájer, 2004; Lugasi, 2007; Yoo et al., 2010).
According to today's understanding, the moderate and regulated consumption of wine has a positive effect on the cardiovascular, digestive, central, and peripheral nervous systems (Antoce & Stockley, 2019; Teissedre et al., 2018). It has been reported that wine consumption has an inverse association with colorectal cancer and light wine intake could protect even against non-alcoholic liver diseases (Kerr & Greenfield, 2007; Dunn et al., 1947). The polyphenol content of wine may be the primary reason for the effects in these studies (Newcomb, 1993). Most of these potentially bioactive compounds are found in grapes, majorly in red grape varieties (Anderson et al., 2005). For example, anthocyanins found in Cabernet Sauvignon, Syrah or in Merlot are effectively extracted into wine (Romero-Cascales, 2005).
This can especially be said about red wines, mainly grown in the Miniș-Măderat wine region. One of the red wine varieties very popularly grown here is the Cabernet Sauvignon (Balla, 2003).
Cabernet Sauvignon comes from southwestern France and is one of the world's most widely recognized and cultivated red wine grape varieties. It is a natural hybrid of Cabernet Franc and Sauvignon Blanc. Cabernet Sauvignon is considered the "king of wines". Even before the phylloxera blight Cabernet Sauvignon was a very popular variety in the Miniș-Măderat wine region. In recent decades, it has become an even more important variety in this wine region.It is due partly to the ease of its cultivation: the grape's thick skin makes it resistant to rotting and the vines are hardy and naturally budding

late so they are more tolerant to frost, and partly to the recognizable character and outstanding quality of the wine made from this grape (Bowers & Meredith, 1997).

In 1979 the area of Cabernet Sauvignon vineyard in the Miniș region was 286 ha, by 1989 it decreased to mere 155 ha. Currently, due to a new wave of popularity continuous replanting takes place in this region (Balla, 2003).

Cabernet Sauvignon has a moderate water requirement and likes a warm climate. Requires long-stem pruning and a lot of canopy management. This variety adapts well in terms of soil, yet produces high-quality fruit on a well-drained gravel soil.

The grapes have a significant pigment content, and the sugar content fluctuates between 208 g/l and 240 g/L, so the wine has a potential alcohol concentration of 12.2–13.9% vol.

The wine is deeply coloured, has delicate fruity aromas, and taste notes reminiscent of blackcurrant, black cherry, or plum. Its darker colour often indicates a higher tannin content (Mihailca & Adam, 1980).

One of the most popularly noted traits of the wine made from this variety is its affinity for oak due to its strong character and high acid and alcohol content. Barrel fermentation softens the grape's naturally high tannins and it also gives the wine unique wood flavours of vanilla and baking spices that complement the grape's typical flavours of black currant, prunes, and tobacco (Dallas et al., 1996).

The other main trait of this variety is that it has a high content of a specific polyphenol called anthocyanin. The phenolic content of wine refers to phenolic compounds, natural phenols and polyphenols, which form a large group of hundreds of chemical compounds that affect the taste, colour and mouthfeel of the wine (DiStefano & Gonzalez-Sanjose, 1991). These compounds include phenolic acids, stilbenoids, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins), and polymers of flavanols (proanthocyanidins). This large group of natural phenols can be roughly divided into two categories: flavonoids and non-flavonoids. Flavonoids include anthocyanins and tannins, which contribute to wine colour and mouthfeel. (Bowser & Meredith, 1997; DiStefano & Gonzalez-Sanjose, 1991).

The grape's phenolic compounds are distributed in various tissues of the berry (skin, pulp and seeds). These phenolic compounds are extracted into the wine during successive fermentation and maceration steps. Depending on the location of the polyphenolic compounds their diffusion into the must-wine and after that their final concentration at the end of the fermentation process can be different. (Pérez-Navarro et al., 2018).

Several studies have investigated the effect of the duration and temperature of maceration on red wine and most importantly on anthocyanin composition (Gil-Muñoz et al., 1999; Gómez-Míguez et al., 2007; Budic-Leto et al., 2008). Amerine (1955) found both colour and flavour were best at temperatures of 21°C and 27°C.

In another study, was found that fermentation at a temperature of 30°C could reach higher results regarding the colour density and flavour than at 20°C (Girard et al., 1997; Gao et al., 1997). In cases when the colour extraction is not appropriately elevated, fermentation temperatures could be applied (Reynolds et al., 2001). Izquierdo-Cañas et al., (2020), mention that the fermentation temperature can have a significant effect on the volatile compounds. Furthermore, the temperature can have a greater impact on the aroma composition of the wines (Sereni et al., 2020). However, Terpou et al., (2020) concluded that at low temperature pine sawdust could be helpful to produce high-quality sweet wine.

In a different study (Pérez-Navarro et al., 2018), it was found that increasing the fermentation temperature at the end of the process leads to more intense colour and higher concentration of phenolic compounds in Pinot noir wines, but at the same time, there is a chance of an increased volatile phenol concentration as well.

In a review by Şener (2018) it was stated that the duration and the temperature of maceration significantly influence the wine's quality and higher temperatures increase the extraction of phenolic compounds. However, fermentation at a lower temperature leads to delayed fermentation, which is a reason for a higher concentration of total phenolic compounds.

The aim of the present study was to determine the influence of changes in fermentation temperature and duration on the anthocyanin content and quality of the resulting wine when using the technology of red wine production with skin fermentation of Cabernet Sauvignon grape variety.

MATERIALS AND METHODS

The experiment was carried out in 2020 at Wine Princess S.R.L., 26 km far from Arad, in Păuliș. Păuliș has been one of the most significant settlements of the Păuliș (Miniș) wine region since the 17^{th}-18^{th} centuries. The vineyard's area is of approximately 70 ha, on which various vine varieties can be found: Cabernet Sauvignon, Cabernet Franc, Kadarka, Pinot Noir, Merlot, Tramini, Muscat, Fetească regală, Italian Riesling. The storage capacity is 5000 hl. The wines are stored in wine cellars dug into the hillside, some of them in barrels, others in bottles depending on the technology involved.
The experiment started with the harvest on the 20^{th} of September 2020 and continued until the 17^{th} of March 2021.
Considering the climatic characteristics, the temperature did not show extreme values in this duration. Negative temperatures could be measured until March, so the sprouting of the grape buds was undisturbed. The average annual temperature was 12.1°C, and the average temperature of the vegetation period was 17.6°C. The absolute maximum temperature was 35.6°C, measured in August, while the absolute minimum temperature was −15°C measured in January.
In terms of precipitation, the average amount of precipitation was 883.8 mm. During the vegetation period, the average precipitation was 559 mm. The average annual air humidity was 73%, and 71% during the growing season.
Grape processing and fermentation
After measuring the quantitative and qualitative properties, the samples were randomly taken from 17,000 kg of grapes. Sugar content was measured with a refractometer and titratable acid content by titrating the samples.
Two variants were separated: Variant 1 (V1) was fermented at 25°C for 10 days, and Variant 2 (V2) at 30°C for 15 days. After the fermentation ended, the sugar content was measured and then the free-run wine was separated and the marc was pressed at a maximum of 1.5 bar pressure. The free-run wine and the press wine were blended in a proportion of 80/20. The quantity of wine produced was 11,050 litres.
After the malolactic fermentation on December 20–21, the first organoleptic examination took place and when the wine's alcohol, titratable acid, volatile acid, free sulphur dioxide content, pH level, and anthocyanin content were determined.
These measurements were made with the multiparameter analyser OenoFoss (FOSS, Hilleroed Denmark), which can instantly measure all the important parameters (ethanol, total acidity, malic acid, lactic acid, volatile acid, glucose, pH, density) from only a few drops of wine, approx. 1–2 mL.
The new wine was poured into medium toast barrique barrels. After three months of barrique fermentation the organoleptic and laboratory examinations were repeated with both variants.
For the evaluation, a simplified 100-point evaluation system was used, issued by the International Organisation of Vine and Wine (O.I.V., 2007). The colour, clarity, intensity (which is determined by the amount of anthocyanin), aroma and bouquet, as well as the taste, overall impression and harmony were examined.
An average was calculated from the results obtained during the evaluation. The objectivity of wine judging is ensured by statistical results. We processed the collected data, treated the organoleptic evaluations of the wine judges as repetitions and recorded them in a computer database.
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 $p < 0.05$.

RESULTS AND DISCUSSIONS

Alcohol content

There is no difference between the variants fermented for 10 days at 26C and the variants fermented for 15 days at 30°C (Figure 1). Cabernet Sauvignon V1 and the Cabernet

Sauvignon V2 were measured on the 20th of December with an alcohol content of 14.1% vol. volume. Since the alcohol content is primarily determined by the amount of sugar accumulated in the grapes, the temperature and duration of fermentation do not affect the alcohol content.
It can be determined that the results from the 15th of March, measured after three months of ageing, do not show any significant differences either. In the case of V2, a change in alcohol content of 0.1% vol. () was observed during the second analysis. This slight increase in alcohol is attributed to residual sugar after fermentation, which was converted to alcohol during malolactic fermentation.

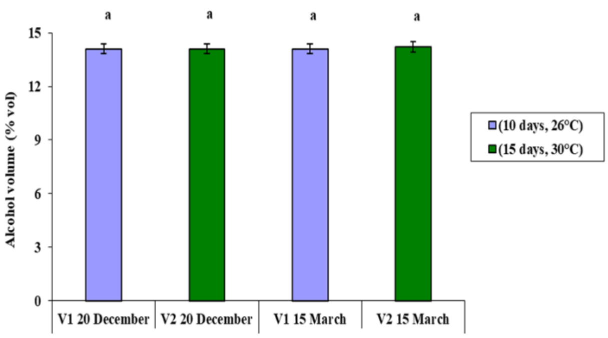

Figure 1. Changes in alcohol content. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on titratable acidity

Examining the titratable acid content (Figure 2), the results showed differences between the variants, however no statistically significant differences were observed. The titratable acidity of the wine fermented for 10 days at 26°C was 7.2 g/L tartaric acid, while the titratable acidity of the wine fermented for 15 days at 30°C was 7.6 g/L tartaric acid. The measurement was made on the 20th of December. The analysis on the 15th of March gave the following titratable acidity results: V1 6.8 g/L tartaric acid and V2 7.2 g/L tartaric acid. It can be determined that there is a difference between the two variants even after three months of ageing. The variant fermented for a longer time and at a higher temperature has a higher titratable acid content. 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.

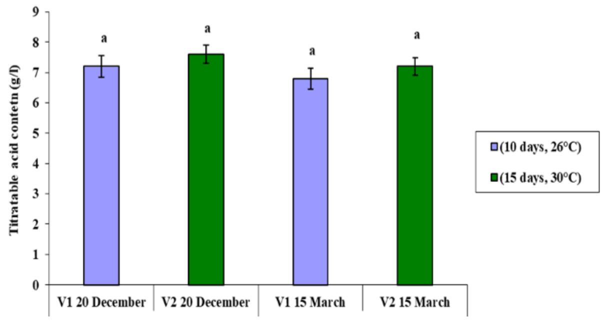

Figure 2. Changes in titratable acid content. Different letters indicate significant differences between treatments ($p < 0.05$).

The effect of changes in fermentation temperature and duration on the volatile acid content

The results of volatile acid content differ between the V1 and V2 variants (Figure 3). The first laboratory test gave the following results: 0.6 g/L acetic acid for wine fermented for 10 days at 26°C, and 0.57 g/L acetic acid for wine fermented for 15 days at 30°C. March results: V1 0.61 g/L acetic acid, V2 0.59 g/L acetic acid. The results also reported that the volatile acid content of both variants increased by 0.01 and 0.02 g/L acetic acid during the three-month maturation period.

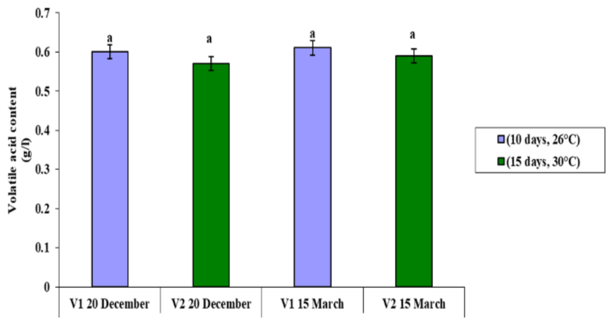

Figure 3. Changes in volatile acid content. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on free sulphur dioxide content
During the examination of the free sulphur dioxide content (Figure 4), it can be determined that the V1 shows a higher value than the V2. Furthermore the 10 days of fermentation at 26°C recorded statistically significant differences, when the two periods were compared. Their values after the December analysis: 27 mg/L for V1 and 21 mg/L for V2. After three months of maturation, the free sulphur dioxide content also remained different considering the two variants.

23 mg/L for wine fermented for 10 days at 26°C and 21 mg/L for wine fermented for 15 days at 30°C. Examining the V1, a decrease of 4 mg/L can be observed during storage, while the free sulphur dioxide content did not change in the case of the V2.

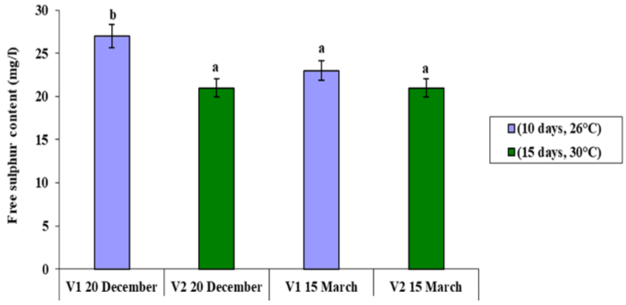

Figure 4. Changes in free sulphur dioxide content. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on pH content

The pH contents (Figure 5) were between 3.1 and 3.3. During the December test, a pH of 3.3 for V1 and 3.1 pH for V2 was measured.. March results were 3.2 and 3.1 pH. in the case of the Cabernet Sauvignon V1, a pH drop of 0.1 was observed during the maturation period, and in the case of V2, the pH content did not change during storage either. No statistically significant differences were recorded.

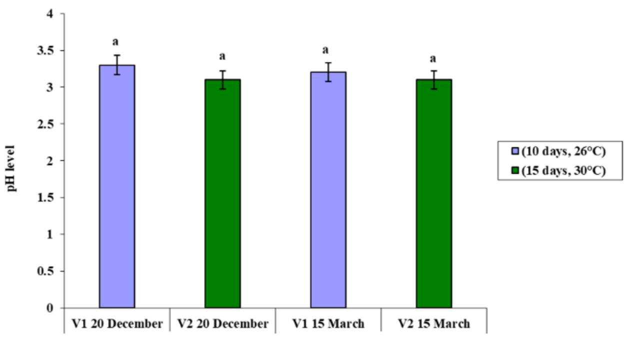

Figure 5. Changes in pH level. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on anthocyanin content

There is a difference between the V1 and V2 in terms of the anthocyanin content, one of the significant factors determining the colour of red wines (Figure 6). The anthocyanin content of the wine fermented for 10 days at 26°C was 420 mg/L, while the anthocyanin content of the wine fermented for 15 days at 30C was 485 mg/L after malolactic fermentation. The results from March were the following: Cabernet Sauvignon V1 with 400 mg/L and V2 with 453 mg/L. The anthocyanin content of the variant fermented at a higher temperature and for a longer time was higher and showed a decrease of 32 mg/L during maturation. The decrease can also be attributed to the anthocyanin content of Cabernet Sauvignon V1. The anthocyanin content of the V2 showed a higher value than the V1, despite the greater decrease. During ripening, highly reactive anthocyanins form anthocyanin-tannin complexes with tannins, as a result of which the anthocyanin content decreases. In the case of our experiment, the decrease in anthocyanin content during storage can also be attributed to this.

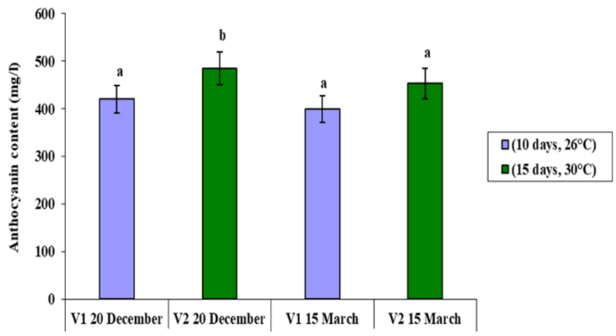

Figure 6. Changes in anthocyanin content. Different letters indicate significant differences between treatments ($p < 0.05$).

Results of the organoleptic examination

From the results of the first sensory test (Figure 7), V2, fermented for a longer time and at a higher temperature, received a higher evaluation. The colour parameter significantly influenced the total score. Since the anthocyanin content of Cabernet Sauvignon V2 was higher, the colour and clarity parameters also had a higher average than in the case of the wine fermented for 10 days and at 26°C. The averages of the organoleptic evaluation of the other parameters were similar, and a very slight difference (1 point) can be seen in the scoring of the aroma. The total score: V1 was evaluated by the judges at 80 points and V2 at 86 points.

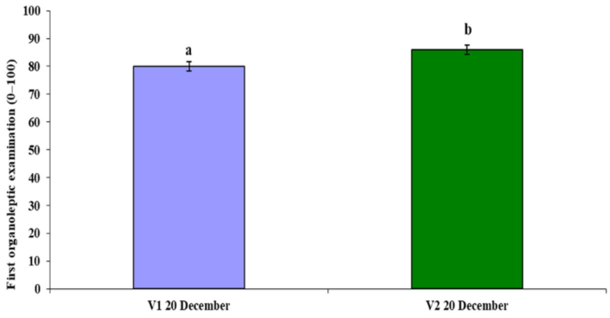

Figure 7. Ratings from the first organoleptic examination.

On the occasion of the second examination on the 15th of March a greater difference in the total score between the variants can be seen compared to the first organoleptic examination (Figure 8).

During ageing in barrels, the intensity of the colours decreased, which resulted in a decrease in the anthocyanin content, but the quality of the colours improved, which is also shown by the assessment of the March test. For both variants, the colour and clarity parameters received more points during the December evaluation. At the same time, it can be seen that there is a 4-point difference between the Cabernet Sauvignon V1 and V2 variants, in terms of the colour parameter. The V2 proved to be better, receiving a 25-point rating. During storage, in addition to the aroma, the bouquet of the wines developed, this factor was also evaluated by the inspection committee. These values also turned out to be better than the first organoleptic test, and here again, the values of V2 were higher.

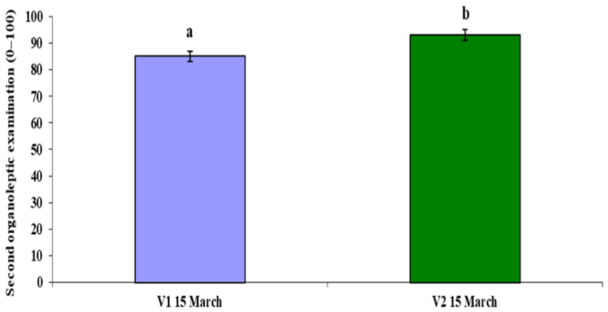

Figure 8. Ratings from the second organoleptic examination.

Anthocyanins are water-soluble vacuolar pigments, and they are responsible for the initial colour of red wines (Kong et al., 2003), furthermore they are strong antioxidants and their quantity is activated under different stress conditions, like extreme temperature, bacterial and fungal infection (Silva et al., 2017). According to Medina-Plaza et al., (2020), the anthocyanin concentration is significantly influenced by a higher temperature and the ethanol concentration. In another study they concluded that the kinetics of anthocyanin adsorption and desorption are clearly dependent on the temperature, ethanol concentration and also cell wall materials composition (Medina-Plaza et al., 2019).

CONCLUSIONS

After examining the obtained results, we can conclude the following: none of the measured parameters (alcohol, titratable acid, volatile acid, free sulphur content and pH level) was affected by the temperature of the fermentation. The anthocyanin level was higher with a higher temperature. In the case of V2, the anthocyanin content was 485 mg/L after 15 days with a fermentation temperature of 30°C. Moreover, lowering of the anthocyanin level while fermenting did not change the quality of colour, only its intensity. The wine had a deeper colour after 3 months of fermentation in the barrique barrels.

Concerning the results of the organoleptic examination of Cabernet Sauvignon, it can be stated that the wine fermented for a longer time and at a higher temperature (Cabernet Sauvignon V2) proved to be better (93 points). A higher fermentation temperature (30°C) and a longer fermentation (15 days) are recommended for this variety in the Miniș wine region. At this temperature and duration during fermentation on marc, the colouring materials and aroma and fragrance materials are dissolved in the appropriate amount from the skin cells of the Cabernet Sauvignon grape.

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MODERN AND CURRENT CHEMICAL ANALYZES OF WINES FROM LOCAL AND AUTOCHTHONOUS QUALITY GRAPECULTIVARS IN THE AMPELOGRAPHIC COLLECTION OF SCDVV DRĂGĂŞANI

Ion BOŞTINARU[1], Sergiu-Ştefan GORJAN[2], Anca BECZE[3], Iulia TOROK[3]

[1]University of Craiova, Faculty of Horticulture, Doctoral School of Animal and Plant Resources Engineering (IRAV), A.I. Cuza Street, no. 13, Zip Code 200585, Craiova, Dolj, Romania
[2]Research and Development Station for Viticulture and Vinification Drăgășani, Regele Ferdinand Blvd, no. 64, Zip Code: 245700, Drăgășani, Vâlcea, Romania
[3]INCDO-INOE 2000 Subsidiary ICIA, Research Institute for Analytical Instrumentation, ICIA, Donath Street, no. 67, CP 717, OP 5, 400293, Cluj-Napoca, Romania

Corresponding author email: ionutbostinaru@yahoo.com

Abstract

The research and analyzes were carried out in 2020-2022 at SCDVV Drăgăşani in the ampelographic collection, and at the Cluj Napoca wine analysis laboratory. The wine grape cultivars analyzed and studied were 'Crâmpoşie', 'Romanie', 'Slaviţă', 'Teişor', 'Bătuta neagră', 'Negru mare', 'Negru vârtos', 'Negru Românesc', grape cultivars that are part of the national collection of germplasm of the Drăgăşani unit. The polyphenols, ascorbic acid, tartaric acid and heavy metals present in the wine were determined, both in the treated and untreated wines of these studied cultivars. Statistical calculations were performed for these wines using statistical indices such as: mean, variance (s2), standard deviation (s) and coefficient of variation (CV %). Statistical significance was determined using the "Multiple comparison test-Tukey Multiple Range Test Procedure (p<0.05)" method. The analyzes performed have shown that these wines are of high quality, the heavy metals in the wine do not exceed the normal limits, so these wines can be successfully classified as DOC and IG wines.

Key words: *ampelographic collection, local grape cultivars, polyphenols, wine analysis laboratory, DOC and IG wines.*

INTRODUCTION

The Research and Development Station for Viticulture and Vinification Drăgăşani is part of the area of the Drăgăşani vineyard, the oldest vineyard in Romania also called the great-grandmother of viticulture. The research station is the first research station in the country founded in 1936.

The most famous and important varieties created at this research station are the varieties for table grapes 'Victoria' and 'Azur', the varieties for white wines 'Crâmpoşie Selecționată' and 'Vilarom' and for red wines the most important are 'Negru de Drăgăşani', 'Novac' and 'Alutus'.

The viticultural research station owns an Ampelographic Collection which is a national viticultural heritage, in the composition of which there are approximately 300 varieties. The varieties taken in this study are found in this collection, they are autochthonous varieties, some even local, with a very good adaptability to the eco-pedological conditions of the vineyard (Teodorescu et al., 2021).

The qualities of the wine obtained from these varieties were highlighted, through physical-chemical analyses, in order to obtain DOC and IG wines and to promote them on the national and international wine market (Popa, 2019).

The Drăgăşani wines therefore continue their traditional performance even today, that of dominating the market with their famous and unparalleled wines.

The Drăgăşani wines enjoy great fame in the country and abroad, being appreciated among the best red wines in the world (Măcău I. & Gorjan S.Ş., 2016).

MATERIALS AND METHODS

The research and analyzes were carried out in 2020-2022 at SCDVV Drăgăşani in the Ampelographic Collection, the Wine Analysis

Laboratory within the S.C. Sâmbureşti domains and the Analysis Laboratory come from Cluj-Napoca. The varieties analyzed and studied were 'Cârlogancă', 'Românie', 'Slaviţă', 'Teişor', 'Bătută neagră', 'Negru mare', 'Negru vârtos', 'Negru Românesc', varieties located within the national germplasm collection of the unit from Drăgăşani.

The grapes of these varieties were vinified, obtaining treated and untreated wines. The physico-chemical analyzes of the wine (performed in 3 repetitions in three years) were performed on the untreated wines as well as on the treated wines. During the wine fermentation process, selected yeasts of 0.2 g/liter and enzymes in the amount of 0.03 g/liter were added to the wines proposed for treatment, in order to stabilize and make compare and determinations. The treatment of the wines proposed for treatment was carried out after carrying out the pritroch and filtering it, with sulfur dioxide in the amount of 1 ml/liter. The second step was carried out with the filtration of the wine and the bentonite treatment was carried out in the amount of 2 grams per liter for the white wine and 1 gram per liter for the red wine. After these operations, the wine was filtered and bottled in 0.750 ml bottles for analysis.

Treated and untreated wines were analyzed, performing chemical analyzes to determine polyphenols, ascorbic acid, malic acid, tartaric acid as well as modern heavy metals present in the wine. For the determination of metals, the Perkin Elmer inductively coupled plasma mass spectrometer, type ELAN DRC II, with quadrupole and equipped with a reaction cell for the elimination of interferents, with a mass range of 3-240 m/z, resolution below 1 amu, (IU -36, Series Q1970307H). Calibrate the device using standard calibration solutions prepared from stock solution (Multi-element Calibration Standard 3, Perkin Elmer).

The obtained data were analyzed with the help of statistical indices such as: mean, variance (s^2), standard deviation (s) and coefficient of variation (CV %). Statistical significance of differences was analyzed using analysis of variance (ANOVA) and *Tukey Multiple Range Test ($p<0.05$)*.

RESULTS AND DISCUSSIONS

Analyzes, observations and determinations were made on these wines from these varieties, both treated and untreated, in order to see the existing qualitative differences, regarding the structure of polyphenols and ascorbic acid (acw) existing in wines.

In Figures 1 and 2 for polyphenols and for ascorbic acid (acw) we have the following determinations:

When determining the average polyphenols in wines during the study period 2020-2022, it can be observed that in most wines there are differences between treated and untreated wines, higher values being found in untreated wines, but from the qualitative point of view of the composition of the wines treated shows, as usual, good values in order to obtain quality white and red wines.

Therefore, for the 'Cârlogancă' wine, we have 116.26 mg/ml echiv GAE for the untreated wine compared to 136.10 mg/ml echiv GAE for the treated wine, the average/variety being 126.2 mg/ml echiv GAE.

In 'Românie' wine, the polyphenol values are 250.22 mg/ml echiv GAE in the untreated wine and 81.96 mg/ml echiv GAE in the treated one, with an average/variety of 161.1 mg/ml echiv GAE.

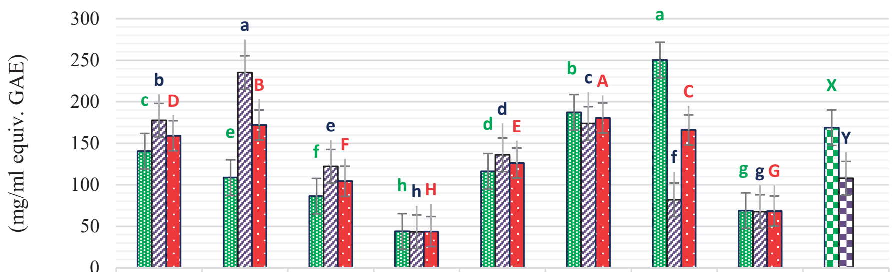
The wine 'Slaviţă' has a value of 68.91 mg/ml echiv GAE for the untreated wine, 67.83 mg/ml echiv GAE for the treated wine, the average/variety being 68.4 mg/ml echiv GAE.

The average value of polyphenols in 'Teişor' wine shows the following values, slightly higher in the untreated wine 43.88 mg/ml echiv GAE compared to the treated wine 43.56 mg/ml echiv GAE with an average/variety of 43.7 mg/ml echiv GAE.

For red wines of the 'Bătută neagră', we have a value of 235.12 mg/ml echiv GAE for the treated wine, compared to 108.71 mg/ml echiv GAE for the untreated wine, so after the treatment of the red wine, the value of the polyphenols in this wine increases. The average/variety is 171.9 mg/ml echiv GAE.

We find the same situation with the 'Negru vârtos' wine, which has a higher value for the treated wine of 122.30 mg/ml echiv GAE compared to the untreated wine, which has a value of 86.27 mg/ml echiv GAE, with an

average/variety of 104.3 mg/ml echiv GAE. The increase in polyphenols in the treated wine is evident in this variety.
A similar situation as with the 2 varieties of red wine presented above, we also have it at the wine 'Negru Românesc' with a value of 177.74 mg/ml echiv GAE for the treated wine compared to 140.42 mg/ml echiv GAE for the untreated wine, the average/variety being 159.3 mg/ml echiv GAE.
In the analysis of the 'Negru mare' wine we have a higher value for the untreated wine of 187.23 mg/ml echiv GAE, compared to the treated wine which has a value of 173.85 mg/ml echiv GAE. The average/variety is 180.5 mg/ml echiv GAE.
We can observe that the average values of polyphenols in the wines studied for these varieties are higher in the case of white wines, in untreated wines compared to treated ones. In the case of red wines, we have higher values of polyphenols in the treated wines compared to the untreated ones, with the exception of the wine from the 'Negru mare' variety, which has a higher value in the untreated wine compared to the treated wine.
We can observe differences in white wines compared to red wines, which have lower values in the constitution of polyphenols, both in treated and untreated wines. The 'Cârlogancă' wine has a higher average value in terms of polyphenols constitution, closer to red wines.
The statistical calculations for these wines, in the determination of polyphenols, indicate that there are significant differences in all the analyzed wines. Measurements are compared to a gallic acid calibration curve (25, 50, 100, 250 ppm) and results are expressed in gallic acid equivalents (Figure 1).



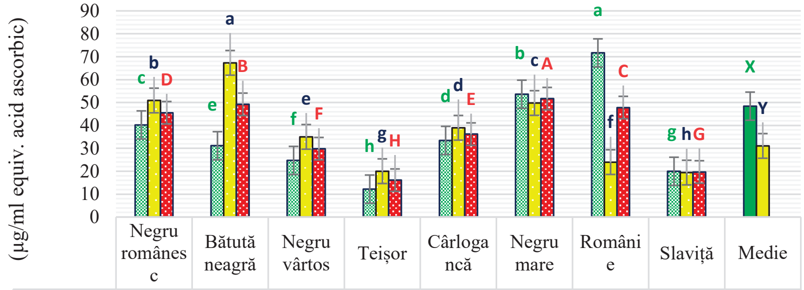
	Negru românesc	Bătută neagră	Negru vârtos	Teişor	Cârlogancă	Negru mare	Românie	Slaviță	Medie
netratat	140,42	108,71	86,27	43,88	116,26	187,23	250,22	68,91	168,8
tratat	177,74	235,12	122,30	43,56	136,10	173,85	81,96	67,83	107,9
Medie soi	159,1	171,9	104,3	43,7	126,2	180,5	166,1	68,4	

The difference between any two variants followed by a common letter is not significant, according to the test *post-hoc* Tukey (p <0,05). The vertical lines represent error of the average (±sE).

Figure 1. Wine chemical analyzes (2020-2022 average) - Poliphenols

The analyzes and determinations carried out regarding the average ascorbic acid (acw) existing in the wines, during the study years 2020-2022, indicate that in five treated wines out of eight, the content of the wine has more ascorbic acid (acw) compared to the wines untreated.
Thus, in the white wine from the 'Cârlogancă' variety, we have 38.97 µg/ml echiv acid ascorbic in the treated wine compared to the untreated wine, which has an ascorbic acid (acw) value of 33.44 µg/ml echiv acid ascorbic, the average/variety being 36.2 µg/ml echiv acid ascorbic.
The white wine from the 'Teişor' variety has 20.01 µg/ml echiv acid ascorbic in the constitution of the treated wine, while the untreated wine has a value of 12.20 µg/ml echiv acid ascorbic. The average/variety has the value of 16.1 µg/ml.

We also have a higher ascorbic acid (acw) content in the treated wines than in the untreated wines, as follows:
The wine from the 'Negru Românesc' variety has the value of ascorbic acid (acw) for the treated wine of 50.89 µg/ml echiv acid ascorbic and for the untreated wine of 40.20 µg/ml echiv acid ascorbic, the average/variety being 45.5 µg/ml echiv acid ascorbic.
We find a very large difference in ascorbic acid (acw) in the red wine o the 'Bătută neagră' variety with an ascorbic acid (acw) value of 67.32 µg/ml echiv acid ascorbic in the treated wine, compared to the untreated one with a value of 31.12 µg/ml echiv acid ascorbic and the average years/variety 49.2 ml echiv acid ascorbic In the red wine of the 'Negru vârtos' variety,we have higher value of 35.01 µg/ml echiv acid ascorbic in the treated wine compared to 24.70 µg/ml echiv acid ascorbic in the untreated wine. The average/varieties 29.9 µg/ml echiv acid ascorbic.
We have a higher content of ascorbic acid (acw) found in the constitution of the wine in the untreated red wine from the 'Negru mare' variety with a value of 53.61 µg/ml echiv acid ascorbic compared to the treated wine which indicates a value of 49.78 µg/ml echiv acid ascorbic, the average of the years/variety having the value of 51.7 µg/ml echiv acid ascorbic.
In two white wines, we find a higher value in the untreated wine, in ascorbic acid (acw) compared to the wine treated as follows: The wine from the 'Românie' variety has a value of high ascorbic acid (acw) of 71.64 µg/ml in the untreated wine, the treated wine having the value of 23.98 µg/ml, and the average of the years/variety being 47.8 µg/ml.
A slightly higher value of ascorbic acid (acw) in the untreated white wine is also found in the 'Slaviţă' variety, with a value of 19.97 µg/ml compared to the treated wine 19.42 µg/ml. The average of the years/variety has the value of 19.7 µg/ml.
Therefore, a higher content of ascorbic acid (acw) in the composition of treated wines is found in red varieties, where three out of four wines have higher values.In the case of white wines, we have two varieties that have higher values in the constitution of wines treated with ascorbic acid (acw), and two untreated wines have higher values compared to the treated ones. We can observe that red wines generally present higher values of ascorbic acid (acw) in treated and untreated wines compared to white wines, with the exception of wines from the 'Cârlogancă' and 'Românie' varieties.
After carrying out the test on these wines, in the determination of ascorbic acid (acw), we can observe significant differences in all the analyzed wines (Figure 2).

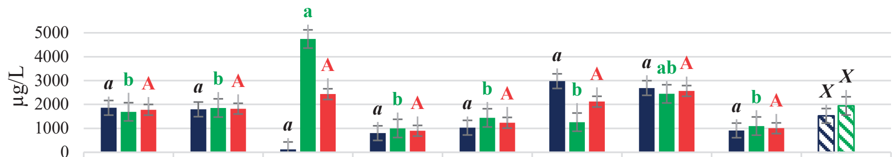


	Negru românesc	Bătută neagră	Negru vârtos	Teișor	Cârlogancă	Negru mare	Românie	Slaviță	Medie
netratat	40,20	31,12	24,70	12,20	33,44	53,61	71,64	19,97	48,4
tratat	50,89	67,32	35,01	20,01	38,97	49,78	23,98	19,42	31,1
Medie soi	45,5	49,2	29,9	16,1	36,2	51,7	47,8	19,7	

The difference between any two variants followed by a common letter is not significant, according to the test *post-hoc* Tukey (p < 0,05). The vertical lines represent the standard error of the average (±sE).

Figure 2. Ascorbic acid ACW (2020-2022 average)

Following the analyzes and determinations made regarding malic acid in the composition of treated and untreated wines, we can observe a higher content of malic acid in five untreated wines. In the treated wines, in number of three we have a higher content of malic acid, in the white wine variety 'Cârlogancă' and in the red varieties 'Negru mare' and 'Negru vârtos' respectively. We generally observe a higher content of malic acid in red wines than white wines, treated or untreated. Through the *Tukey post-hoc* test (p<0,05- means >95% probability) for these wines, treated and untreated, we can observe insignificant differences regarding the malic acid content of the wines according to statistical calculations (Figure 3).

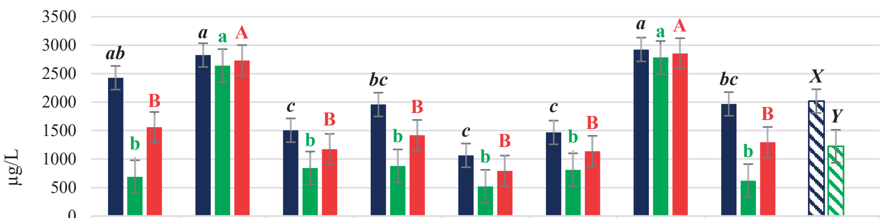


	'Cârlogancă'	'Românie'	Slaviță'	'Teișor'	'Bătută neagră'	'Negru mare'	'Negru vârtos'	'Negru românesc'	Medie
Tratat	1861,07	1796,13	125,02	801,51	1028,99	2977,09	2689,84	915,15	1524,35
Netratat	1693,84	1854,88	4741,87	998,32	1440,40	1261,02	2444,54	1096,47	1941,42
Medie	1777,46	1825,51	2433,45	899,92	1234,69	2119,06	2567,19	1005,81	

The difference between any two variants followed by a common letter is not significant , according to the test *post hoc* Tukey (p < 0,05).
The vetical line s represent the standard error of the average (±sE).

Figure 3. The malic acid content of the analyzed wines, 2020-2022

Regarding the average content in tartaric acid µg/L in these studied wines, we observe that in all the wines treated both in the white and red varieties, a higher content of tartaric acid.
From the analyzes carried out, we find that white and red wines from these varieties have a similar µg/L tartaric acid content. The treated wines from these varieties have a higher content of tartaric acid µg/L compared to the untreated ones. After performing the *Tukey post-hoc* test (p<0.05) we find that there are significant differences between the varieties, namely in the varieties 'Românie', 'Negru vârtos' with a higher content in tartaric acid µg/L compared to the other varieties (Figure 4).



	'Cârlogancă'	'Românie'	Slaviță'	'Teișor'	'Bătută neagră'	'Negru mare'	'Negru vârtos'	'Negru românesc'	Medie
Tratat	2428,08	2826,44	1505,82	1958,11	1065,22	1468,15	2924,89	1968,81	2018,19
Netratat	690,38	2642,16	844,45	880,76	522,46	812,68	2785,28	622,65	1225,10
Medie	1559,23	2734,30	1175,14	1419,44	793,84	1140,42	2855,09	1295,73	

The difference between any two variants followed by a common letter is not significant, according to the test *post-hoc* Tukey (p < 0,05).
The vertical lines represent the standard error of the average (±sE).

Figure 4. The tartaric acid content of the analyzed wines, 2020-2022

Following the determination of the heavy metals in the wines of the analyzed varieties, we observe that they do not exceed the legal norms of major risk in this food.

After carrying out this study we can observe in general that these wines present higher values of heavy metals in untreated wines, these values decreasing after treatment (Table 1).

For the chemical element Pb (µg/L) we find a higher value in the treated wine of the 'Negru Românesc' variety compared to the untreated one, the other wines analyzed showing higher values before the treatment.

In the red wines of the varieties studied, the chemical element Na (µg/L) has higher values in the treated wines than in the untreated ones, which indicate higher values in the white wines.

For the chemical element Ni (µg/L), the untreated wines show higher values than the treated wines, except for the treated wine of the 'Bătută neagră' variety, which shows a higher value.

The chemical element Al (µg/L) has a higher content in treated white wines, and a higher content in untreated wines in red wines.

Table1. Determination of heavy metals in wines analyzed by the ICP-MS method in the period 2020-2022

Element	Variety/Variant/Year	Wine Treat			Wine Untreated		
		Average	*Standard deviation (SD)*	*Coefficient of variation (CV %)*	***Average***	*Standard deviation (SD)*	*Coefficient of variation (CV %)*
Pb (µg/L)	'Cârlogancă'	3.4	0.0	0.00	5.6	0.1	1.79
	'Românie'	1.8	0.1	5.56	2.5	0.1	4.00
	Slaviță'	2.2	0.1	4.55	3.4	0.1	2.94
	'Teişor'	2.7	0.1	3.70	4.4	0.1	2.27
	'Bătută neagră'	2.8	0.1	3.57	3.6	0.1	2.78
	'Negru mare'	2.4	0.1	4.17	3.2	0.1	3.13
	'Negru vârtos'	2.9	0.0	0.00	3.1	0.0	0.00
	'Negru românesc'	2.2	0.1	4.55	1.2	0.1	8.33
Na (µg/L)	'Cârlogancă'	4106	1.0	0.02	5831	1.0	0.02
	'Românie'	4622	1.0	0.02	5278	1.0	0.02
	Slaviță'	4806	1.0	0.02	5252	1.0	0.02
	'Teişor'	4522	1.0	0.02	5678	1.0	0.02
	'Bătută neagră'	4372	1.0	0.02	4250	1.0	0.02
	'Negru mare'	5222	1.0	0.02	5620	1.0	0.02
	'Negru vârtos'	5458	1.0	0.02	5952	1.0	0.02
	'Negru românesc'	5015	1.0	0.02	4448	1.0	0.02
Ni (µg/L)	'Cârlogancă'	5.1	0.1	1.96	9.4	0.1	1.22
	'Românie'	9.8	0.1	1.02	14.2	0.1	0.70
	Slaviță'	19.4	0.1	0.52	22.2	0.1	0.45
	'Teişor'	7.8	0.0	0.00	10.6	0.1	0.94
	'Bătută neagră'	16.8	0.0	0.00	16.1	0.0	0.00
	'Negru mare'	17.8	0.1	0.56	20.6	0.1	0.56
	'Negru vârtos'	17.8	0	0	19.1	0	0
	'Negru românesc'	17.4	0.1	0.57	20.4	0.1	0.49
Al (µg/L)	'Cârlogancă'	537.5	0.1	0.02	443.5	0.1	0.02
	'Românie'	522.4	0.1	0.02	514.6	0.1	0.02
	Slaviță'	490.6	0.1	0.02	422.5	0.1	0.02
	'Teişor'	524.9	0	0	482.8	0	0
	'Bătută neagră'	58.5	0.1	0.17	72.5	0.1	0.14
	'Negru mare'	82.5	0.1	0.12	98.4	0.1	0.10
	'Negru vârtos'	89.5	0.1	0.11	104.5	0.1	0.10
	'Negru românesc'	28.6	0.1	0.35	39.5	0.1	0.25

CONCLUSIONS

The wines obtained from these varieties of a certain value meet all the conditions for obtaining quality white and red wines.

The polyphenols, ascorbic acid, malic acid and tartaric acid have a decisive role in obtaining the quality of these wines.

Heavy metals existing below the legal norms in these wines prove that they are not a danger to human health and thus, a moderate consumption of wine does not have a negative impact on human health.

In the future, the aim is to promote these old, local wines from the ampelographic collection of SCDVV Drăgăşani on the wine market.

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OPPORTUNITIES FOR ENSURING THE PROFITABILITY OF A SMALL WINEMAKING HOLDING IN ROMANIA

Georgeta Mihaela BUCUR[1], Gabriela MATEI[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Ministry of Agriculture and Rural Development, 24 Carol I Avenue, District 3, 020291, Bucharest, Romania

Corresponding author email: gabriela.matei.office@gmail.com

Abstract

The paper aimed to identify the opportunities to ensure the profitability of a small wine holding of 5.15 ha in Romania. It is based on the analysis of good practice models from Romania and Europe, as well as an economic and financial analysis of the current situation of the holding, together with a market study to identify the preferences of Romanian consumers in the field of wine tourism, culminating in exploring funding opportunities through the Common Agricultural Policy (CAP) in Romania for the period 2023-2027. Research shows that the profitability of wine production on a small wine-growing depends to a large extent on the visibility of the product, and wine tourism is the most effective way. Wine tourism is already known among romanians, with 44% of respondents having already experienced wine tourism home and abroad. As a conclusion, supporting a small-scale wine-growing holding implies, firstly, financial resources and, secondly, the development of complementary income-generating activities that increase the visibility of the agri-food products. Therefore, limited-edition quality wine can generate profits comparable to large wineries with adequate funding and the right image policy.

Key words: *winemaking, small vineyard, CAP in Romania, wine tourism, business profitability.*

INTRODUCTION

Romania's current socio-economic situation shows a significant decline as a result of the crisis caused by the SARS-CoV-19 pandemic, which is also confirmed by the data provided for the HoReCa (Hotel/Restaurant/Catering) industry. In 2021, the most affected wine sales channel was the hospitality segment, which, compared to the same period of the previous year, suffered a 90% loss of wine sales, according to the "Best Wine Importers" database (www.bestwineimporters.com).

The effects of the pandemic are not the only limiting causes of the vine and wine industry, the Russo-Ukrainian War also causes numerous economic imbalances generating alarming price increases and, implicitly, higher production costs in the agri-food sector. All these imbalances, at national level, are also intensified by a growing shortage of specialised staff, but also of agricultural workers. Climate change, in conjunction with the difficulties of small romanian holdings to move towards the market, the imbalances between imports and exports existing in Romania, determine more and more farmers, including wine growers, to abandon their agricultural areas, focusing on other income-generating activities.

In this context, the paper aims to identify solutions to ensure the profitability of a small wine holding of 5.15 ha, of which only 3 ha are owned; a family business which currently sells only wine grapes, its profitability being even below subsistence level with a ratio of income to expenditure being less than 6000 Euro annually.

Market research on small businesses shows that in order to achieve a higher-than-average level of performance, SMEs aim to occupy the ground through large-scale economic consolidation and development or make a difference through quality or innovation (Newton et al., 2015; Ageieva & Agarkova, 2018). Thus, an increasing number of winegrowers are expanding their agricultural business towards the diversification of marketed products (e.g. grape seed oil, grape juice, food supplements made from grapes), including ideal conditions for tourism, but also leisure activities (Nemethy et al., 2016). There are three groups of tourism services/products in a wine-growing region: the 'basic product' referring to wine itself, 'augmented services' covering all services and activities under the control of the winery, such as wine-growing activities, customer services and social events or wine clubs, and ultimately 'ancillary services' refer to services and activities that are mostly out of the winery's control, including other regional tourist activities, local entertainment, accommodation and transport. The basic benefits like wine tasting or wine purchase, seem to be the most important factors for attracting tourists to a wine region or a specific winery (Byrd et al., 2016).

However, the uniqueness of a small business does not lead to differentiation unless it is valuable to a buyer, and the basis for differentiation is the role of the product offered by a small enterprise in the market value chain in line with the buyer's needs (Porter, 1985).

A model of good practice in France, which demonstrates that it can meet the needs of consumers by adding innovative elements for the success of a small wine business, is the *Barbossi Domain*, which operates an area of 4 ha, planted with the grape varieties Cinsault, Grenache, Syrah, Chardonnay and Muscat, offering consumers wines with designation of origin and blends. Since 2021 the replanted vine areas have become organic certified and the wines are produced in accordance with the organic winemaking rules, so the owners are geared towards giving all the necessary conditions to obtain exclusive Grand-Cru wines in limited quantities at appropriate prices.

In terms of complementary activities, the *Barbossi Domain* offers accommodation in a 4-star hotel "Ermitage de l'Oasis" and gives a bottle of wine for the guests, as well as invitations to free wine tasting sessions. It also organises a series of events that include: culinary associations with the produced wines; wine presentations, sports activities (tennis, riding, golf), parties in the vineyard, thus covering a wide range of consumer needs. They also have a store with traditional products obtained from agri-food activities such as honey and olive oil, products have local specifics (www.domainedebarbossi.fr).

This business model reached a turnover of EUR 681 800 in 2019 and EUR 496 700 in 2020 for the commercial and wine-growing holdings. The decrease in turnover is caused by the losses generated by the crisis caused by the SARS-CoV-19 pandemic, but the investments in re-improvement of the services offered alongside marketing and promotion techniques show the sustainability of this business and the development of promotion in the online environment.

In Romania, there are few successful models for small wine businesses, but those that exist demonstrate that it is possible to develop a small wine holding for a real profitable business (Neacșu, 2012). About the pioneers of these types of wine cellars, we mention: Ferdi Winery (4.5 ha) in Dealu Mare Wine Region; Cote Winery (2 ha) in Cotesti Wine Region and Gabai Winery (3 ha), in the region of Dobrogea Hills.

Ferdi Boutique Winery, founded in 2006, owns 4.5 ha of vineyards in the Wine Region Dealu Mare (Ceptura and Vulcănești). The winery's vision involves tradition, crafts and exclusivity, and the wines produced are obtained from the following varieties: Fetească neagră, Merlot, Syrah, Tămâioasă românească and Sauvignon blanc. As a marketing strategy, the winery carries out tasting activities (standard, premium and private); but it also offers accommodation facilities through the collaboration with KIM Country Club (Premium Team Events SRL), which allows access to the restaurant, the swimming pool and the organisation of wine tastings, also offering the possibility to organise events in the location (www.cramaferdi.ro, www.kimcountryclub.ro).

Another good prac-tice model in Romania is the *Gabai Boutique Winery* in Murfatlar wineyard, founded in 2014, and which capitalises on an area of 3 hectares with grapevine (Muscat Ottonel, Riesling italian, Fetească neagră, Pinot noir). The financial situation of the wine cellar shows good profitability before the crisis caused by the SARS-CoV-19 pandemic, reaching a profit of EUR 298 695 in 2018, followed by major decreases in the following years, so that in 2021 the profit reached less than half compared to 2018 (Figure 1).

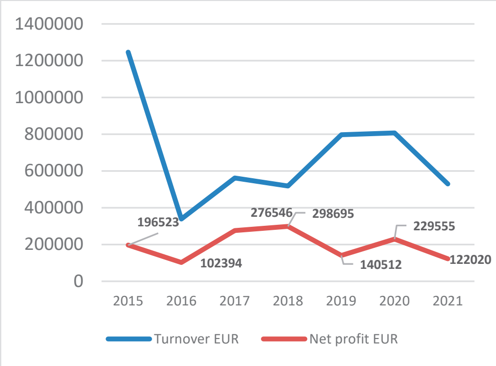

Figure 1. Financial data Winery Gabai, 2015-2021
(source: https://www.listafirme.ro/)

MATERIALS AND METHODS

The study aimed at *identifying alternatives for increasing the viability of a small winegrowing holding,* by proposing an integrated business plan that, together with the activity of obtaining wine grapes, would contribute with added value through wine production, but also by creating oenoturistical and recreational activities and, last but not least, identify innovative business models to access the wine market in Romania and ensure the long-term sustainability and profitability of the holding.

Winegrowing holding location, varieties and cultivated areas. The analysed small wine-growing holding is located in the Odobeşti Controlled Designation of Origin area, in the viticultural region of Moldova, on the ancient domain of the Beciul Domnesc, with easy access to water, electricity, gas and paved agricultural roads. The area owned is 5.15 ha consisting of separate physical blocks cultivated with noble wine grape varieties (Table 1). The sale of grapes and must (on request) is carried out only with the customer's packaging, thus eliminating the costs of packaging the products. In order to identify the best opportunities to ensure the profitability of the small winegrowing holding, a mix of methods was used such as an *economic-financial analysis* and *a SWOT analysis* of the current situation of the holding. The SWOT analysis was used as a method of assessing the performance, competition, risk and potential of a business by identifying the strengths, weaknesses, oppor-tunities, and threats of the current wine-growing holding small business.

Table 1. The structure of the wine farm
- cultivated areas and varieties, 2021 -
(Matei & Bucur, 2022)

Variety	Surface (ha)	Age (years)	Yield (t/ha)	Utilization of the harvest
Muscat Ottonel	1.7	6	10-13	
Riesling italian	1.0	35	6-8	selling grapes and must
Fetească regală	0.2	40	3-5	
Sauvignon blanc	0.8	35	6-7	
Merlot	1.45	35	7-8	

It has also been conducted *a market survey* on consumer preferences for wine and oenotourism carried out on a representative sample of 390 persons, between May and June 2022, with responses collected through the Survey Monkey platform. The questionnaire, entitled "The perception of Romanian consumers on wine tourism" included questions on the age, gender and income of respondents, the willingness to spend time or money to enjoy the wine experience, but also preferences in terms of wine tourism and leisure activities in the wine environment. In order to obtain relevant results, it was spread both in urban and rural areas, on the social media pages of HoReCa entrepreneurs, among corporations, students and teachers, but also among civil servants.

In order to identify the best resources to develop the business, *funding opportunities offered by the post-2020 Common Agricultural Policy (CAP) for Romania* for the proposal of a business plan were also explored.

RESULTS AND DISCUSSIONS

Results concerning the economic and financial analysis of the wine-growing holding. An economic and financial analysis (Table 2), together with a SWOT analysis on the Odobeşti vineyard holding, shows that there is over-production, giving up quality in favour of quantity, in order to obtain a profit, which is extremely low in relation to the operating costs of the 5.15 ha of vines (only 26% of expenditures and 21% of income). Also, technological facilities are precarious and obsolete, wine-growing areas are not merged, which is why there is a loss of income, by moving agricultural machinery and labour from one parcel to another. Also, the profitability of the holding is so low that it could not provide a monthly income to the farmer, the EUR 5 829 collected annually, providing an income of less than EUR 500/month.

The SWOT analysis (Table 3) identified opportunities for financial support such as the availability of European funds dedicated to the wine sector, making it possible to restructure the business in order to increase its profitability, but also to integrate best practice models that aim at alternatives in the diversification of the wine-growing activity. An important strength identified is the involvement of the family members. Thus, the farmer's daughter, who has adequate skills in the agri-food sector, is the most suitable family member who could continue to manage the holding and develop it as a young farmer (34 years old), which becomes an opportunity, next to other opportunities identified like the accelerated expansion of the online environment including marketing and promotion or the access to advanced tech-nologies to make labour costs more efficient and avoid production losses.

All these opportunities can be seized, given the openness of young people to new technologies and to the online environment.

Table 2. Estimate of expenditure and revenue for holding of 5.15 ha (year 2021) (Matei & Bucur, 2022)

Expenses	Details	Value (EURO)
The workforce 12 days/year	team Leader (1) and teams (8)	3600
Agricultural works	manual and mechanised works	16951
Maintenance of machinery	tractor repair	640
Flat-rate tax	annual	60
Marketing	online and newspapers	38
Cost of rent	20 % of the harvest	1126
Total expenditure		**22416**
Income	**Details**	**Value (EURO)**
Single area payment scheme	Payments and Intervention Agency for Agriculture (PIAA) subsidies	492
Redistributive payment		248
Simplified scheme for small farmers		926
Environment Agricultural Practices		298
Valorization of the harvest	sale of grapes and must	26281
Total income		**28245**
Income-to-expenditure ratio	**+ 5829 EURO**	

Results of the market study in terms of profit alternatives that can be obtained from tourism and leisure activities. The market study undertaken revealed that the range of consumer preferences is wide and the main places are successfully occupied by culinary partnerships, guided tours to wineries, participation in grape picking and must processing for entertainment purposes, and Romanians seem keen to practice these activities. The respondents (1.79%) also provided new ideas like setting up a blending laboratory (Figure 2). Furthermore, over 43% of the 390 respondents have never practised wine tourism but would like to try it, while almost 25% of them would not even want to try. But an aggregation of the main answers shows that about 75% of respondents are interested in oenoturism, and over 44% of them have already practiced it in the country (26%) and abroad (18%). These data confirm the assumption that oenoturistic activities have the potential to be increasingly preferred by consumers. Moreover, given that tourism in-volves both leisure but mainly accommodation, questions were dedicated dedicated exclusively to the interest of accommodation in a weekend destination, and surprisingly, for most respondents (48%) it does not matter the number of hours spent on the road, if the destination is worth visiting.

Table 3. The SWOT analysis of the holding of 5.15 ha
(Matei & Bucur, 2022)

Strengths	Weaknesses
- Location in a wine-growing area of notoriety, loaded with history; - Good quality of the grapes obtained (leads to faithful customers); - Possibility of involving family members in business development; - Holding functional buildings for the harvesting campaign (agricultural workers accommodation and grape storage); - Owning solar panels for energy efficiency.	- Lack of adequate technological equipment for wine production, in order to obtain added value on the product; - Low profitability of the holding maintaining it in the subsistence area; - There are no risk management practices; - Transport and delivery of grapes are not offered; - Difficult access to the wine market.
Threats	**Opportunities**
- Accelerated price increases for agricultural inputs without adequate mitigation measures; - Accelerated climate change effects leading to production losses; - Fiscal and legislative instability at national level; - Delay in the implementation of the Strategic Plan CAP 2023-2027.	- Advanced technologies to make labour costs more efficient and avoid production losses; - Availability of EU funds dedicated to the wine sector; - Increasing visibility of wine tourism; - Accelerated expansion of the online environment including for marketing and promotion.

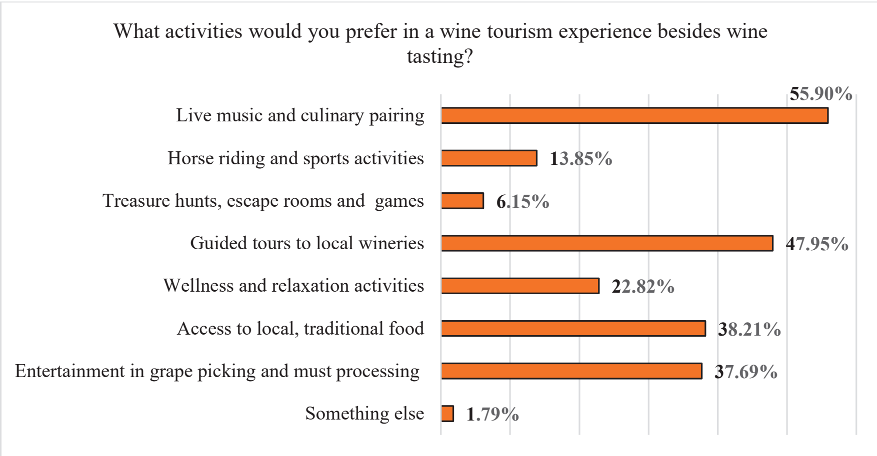

Figure 2. Favourite oenotouristic activities, the results from with the questionnaire during the period May - June 2022 (Matei & Bucur, 2022)

Results on the development of an integrated business plan to ensure the profitability of a small wine-growing holding

Business models represent both the connections between the key components of the business model (the theory building approach) and the transformational tools for addressing change, introducing innovation and competitive advantage in enterprises. Business models therefore serve the purpose of generating new value by anticipating new possibilities, as well as changing, challenging and even defying existing business models within broader socio-technical transitions (Voelpel et al., 2004).

Thus, given the strength identified in the SWOT analysis regarding the possibility of family involvement in farm activities, as well as the opportunity regarding the availability of European funds, the study channelled into an analysis of EU funds available through the CAP Strategic Plan 2023-2027 and which could be accessed by the farmer and his family.

The CAP Strategic Plan 2023-2027 offers a set of interventions for financing agricultural and rural development activities. Every intervention has a particular code and number chosen by the Member State, and the codification is related to the object of the intervention *I-S-V* meaning

“Sectorial Intervention in Viticulture” and *“DR”* meaning *Rural Development Intervention.*

Therefore, a new business plan is proposed with a time horizon of 5 years, starting in the spring of 2023, when the first calls for accessing European funds will be opened. The first intervention to be accessed is "DR-30 - Support for setting-up of young farmers", which will grant in the first phase 75% of the 70 000 EUR, from which a series of essential purchases will be made to eliminate financial losses. Thus, family involvement will be achieved through the setting-up of the farmer's daughter as a young farmer under this intervention. All the details of the investments proposed by the new Business Plan model are shown in Table 4 (for the period 2023-2024) and Table 5 (for the period 2025-2027) and the intervention IS-V-03 *Crop Insurance*, aimed at insuring wine plantations will be accessed annually (with a 80% reimbursement of insurance payments).

In the first phase, the new Business Plan Model focuses on ensuring the performance of the current wine-growing holding.

Table 4. Investments proposed by the new Business Plan model (2023-2024) (Matei & Bucur, 2022)

Year 2023_Funding	**Year 2024**_Funding		
DR-30 Support for setting up young farmers	***DR-17*** Investments in hops and/or table grapes	***DR-34*** Cooperation and innovation in agriculture through EIP Operational Groups	***DR-36*** Leader Community-led local development
The acquisitions:	**Investments:**		
Tractor BCS Volcan K105	Establishment of 2 ha table grapes	Setting up a wine festival	5 accommodation units made of biodegradable materials
A/C machine 24000 BTU	Establishment/modernisation of hygiene and biosecurity facilities at farm level	Purchase of a refrigerated caravan for agri-food freight transport	Purchasing tent events
Working capital	Establishment, extension and modernisation of farm-level conditioning units	Acquisitions of licenses and software	Designing the tent events
Online marketing	Development of the sales /marketing component at farm level (stores at the farm gate)	Construction of a 124 m^3 refrigerated warehouse	Arranging the events space and planting hedges
Amount (100 % EU)	**Amount (65 % EU)**	**Amount (100 % EU)**	**Amount (65 % EU)**
EUR 70 000	**EUR 400 000**	**EUR 300 000**	**EUR 200 000**

The second phase of the business plan involves the orientation towards touristic and leisure services and the third phase of the business plan involves the setting up of table grape vineyards in order to increase the profitability of the holding next to the basis for winemaking. The fourth phase of the business plan involves the construction of a guesthouse and a restaurant within the pension, as well as a wine tasting space, similar to the French best practice model, which supports a cluster of agricultural and non-agricultural businesses in an integrated system that ensures sustainability, visibility and international success.

Thus, by providing a total investment of EUR 2 370 000 of which EU funding represents 71% (EUR 1 685 000), over a 5-year time horizon, there is the possibility of developing the small wine-growing business of 5.15 ha towards competitiveness, innovation, environmentally friendly agriculture practices by increasing resource efficiency and increasing the visibility of the area by creating a large-scale event attracting small producers who want to sell and promote their products, ultimately resulting in the production of quality wine, at international standards with an appropriate visibility for success.

The main risks identified concern difficulties in accessing European support, bureaucratic, administrative and legislative obstacles, delays in launching national calls and delays in approving projects. There is the risk of ex-ante investments for which the amounts invested can no longer be recovered, but all these risks are mitigated by identifying a wide range of financing measures with European funds, entering into partnerships with specialists in the field and flexibility regarding the implementation period of the business plan.

Table 5. Investments proposed by the new Business Plan model (2025-2027)
(Matei & Bucur, 2022)

Year 2025		**Year 2026**	**Year 2027**
IS-V-07 Investments in tangible and intangible assets to increase the sustainabi-lity of wine production	***IS-V-02*** Investments in tangible and intangible assets for the wine sector	***DR-12*** Investments in strengthe-ning holdings of newly in-stalled and newly installed farmers	***DR-24*** Investments in the crea-tion and development of non-agricultural activi-ties
Investments		**The acquisitions**	**Investments**
Procurement of water-saving drip irrigation and fertilisation systems	Construction of buildings intended for winemaking	Precision agriculture equipment, including for the management/use of fertilisers and/or plant protection products	Construction of an agro-pension of 180 sqm, with accommodation capacity 30 persons
Machinery and equip-ment for: application of organic and mineral fertilisers, spraying with low volume of solution	Installations related to re-ception, production, pack-aging, storage	Installations for the local control of hail fall and ground generators	Restaurant hall for wine tasting events
Seeder for sowing the intervals between rows	Setting up a blending laboratory	Production and use of energy (electrical and/or thermal) from renewable sources (solar, wind, aerothermal, hydrothermal, geothermal, etc), intended exclusively for own con-sumption	Installation of solar panels on the roof of the restaurant
	purchase of a pellet production machine from chopped vine ropes		
Amount (50 % EU)	**Amount (50 % EU)**	**Amount (80 % EU)**	**Amount (65 % EU)**
EUR 250 000	**EUR 400 000**	**EUR 550 000**	**EUR 200 000**

CONCLUSIONS

Therefore, the new Business Plan model will include complementary activities that financially support agricultural activities, directing the activities carried out towards producing their own exclusive wine, addressing an educated with financial resources audience, applying a price policy suitable for the majority of respondents participating in the market study. The promotion strategies will also take place in the online environment which will allow the creation of content to promote the various local producers in the area which will then represent a channel for promoting their own products and services of the small wine-growing holding.

In order to start the wine production activity, it will be necessary to contract the services of a wine technologist, who will be co-opted from Australia, to create a limited-edition niche wine similar to the *Ferdi Winery* good practices model.

All the investments are therefore intended to lead the current wine holding towards the production and sale of wine on the market, with the necessary equipment, with the application of appropriate and economically efficient agricultural practices.

The labour cost will be reduced by the installation of metal trellises within the owned wine areas (3 ha) along with the renting of a harvesting machine, but this can only be done after the wine cellar has been established.

The Business Plan model is based on European funds, as the Common Agricultural Policy was set up to mitigate the losses of farmers and it is well known that agricultural activities are not sufficiently profitable and competitive, especially among small farmers. Without substantial financial capital (between EUR 1 000 000 and EUR 3 000 000), one cannot start a business in the agricultural sector, especially in the wine-making field, and without investments to drive businesses towards competitiveness, small farms remain at the subsistence stage, producing at a loss most of the time, agriculture being practiced in many cases from debt to acquired inheritances, or from the specific mentality of Romanians who prefer to be owners on unproductive land, rather than handing them over to farmers with investment power.

The support of a small wine-growing holding involves, first, financial resources and secondly the carrying out of complementary revenue-generating activities that increase the visibility of the agri-food products offered, so that the market orientation is supported by a continuous flow, through relevant partners for promotion and marketing, and last but not least, the creation of own brand that achieves success in sales without the need for additional actions specific to an integrated business plan, by offering high quality wine and creating an attractive image in line with trends in the national and international market.

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RESEARCH CONCERNING THE REACTION OF VINES TO VARIABLE CLIMATIC CONDITIONS

Dorin Constantin COSTEA, Ramona CĂPRUCIU, Daniela Doloris CICHI, Constantin BĂDUCĂ CÎMPEANU

University of Craiova, Department of Horticulture and Food Science, Faculty of Horticulture, 13 A.I. Cuza Street, Craiova, Romania

Corresponding author email: dorin.costea@edu.ucv.ro

Abstract

Among the effects of climate change are the increase in the average annual temperature, the increase in the frequency of extreme meteorological phenomena, the reduction of the amount or the uneven distribution of precipitation during the period of vegetation, periods of drought betwixt the climatic factors, the water regime associated with the ambient temperature are determining environmental factors that affect all aspects of plant growth and development and have the most significant impact on the quality and the quantity of the production obtained. The grapevine has developed response mechanisms to cope with water stress and high temperature. These mechanisms include adaptations of a morphological, anatomical, physiological, biochemical nature that allow the vine to overcome periods of environmental stress but affect the quantity and quality of production. This paper presents the results of the studies on the response of the vines to the variable climatic conditions in the wine-growing area for the production of D.O.C. wines Banu Mărăcine.

***Key words**: climatic conditions, vines, response mechanisms.*

INTRODUCTION

Vitis vinifera (L) is a species sensitive to climate change (Battaglini et al., 2009; Van Leeuwen et al., 2019). The climate traces the dominant characteristics of the wine-growing area, imposes the assortment of varieties and the direction of production (Dobrei et al., 2018; Dejeu et al., 2008; Marăcineanu et al., 2021) Schultz at al., 2010). Ecoclimatic conditions determines the growth and fruiting and gives uniqueness to the grapes obtained in a certain area (Bora et al., 2014; Costea et al., 2015; Gladstones, 1992; Rotaru et al., 2010; Webb et al., 2013). In order to perform durable viticulture, studies were made trying to identify the reaction mechanisms of grapevine under conditions of climate change (Battaglini et al., 2009; Boutin et al., 2012; Prieto et al., 2020). Numerous researches have highlighted the effect of temperature on the intensity of physiological processes, metabolism and on berry composition (Bonada & Sadras 2015; Bucur & Dejeu, 2017; Căpruciu et al., 2022; Costea et al., 2010, Sweetman et al., 2014; Yamane et al., 2006). Among the physiological processes, photosynthesis is considered to be the first process affected by temperature variations. For vines the optimum temperature for photosynthesis is between 25 and 35 °C (Burzo et al., 2005; Xenofon et al., 2020). Higher or lower temperatures have the effect of reducing the intensity of photosynthesis. The grapevine has an internal adaptation mechanism to combat heat stress. Studies on the effects of high temperatures on gas exchange demonstrated that temperatures above 40°C caused a sustained reduction in photosynthesis, which was attributed 95% to a reduction in stomatal conductance (Cichi D.D., 2006; Greer & Weedon, 2012). The reduction of stomatal conductance is one of the first reactions to thermal stress. Most of all, heat stress is often accompanied by seasonal drought stress. Stomatal closure, which also reduces transpiration, is a first defense mechanism against potential desiccation (Burzo et al., 2005; Greer & Weedon, 2012). However, transpiration is irreplaceable. Even a low transpiration rate can cause the leaf temperature to drop by a few degrees, which in some cases is the difference between growth and wilting (Anconelli & Battilani, 2000; Greer, 2020). Weather conditions throughout the year have a greater influence than other factors (such as soil and cultivars) on grapevine development and berry composition (Drappier et al., 2019). Berries produced under water deficit conditions were smaller and characterized by a higher skin-to-flesh ratio (Triolo et al., 2019; Ubalde et al., 2010). The variability of climatic conditions generates potential risks to which winegrowers must respond by adapting the technology of grapevine culture (Neethling et al., 2017). Due to the perennial nature of grapevine culture, adaptation strategies must take into account both the long-term impact short as well as the long term of climate change, based on the specificity of the crop area (Duchene et al., 2010; Leeuwen & Darriet, 2016; Quénol, 2014). The choice of plant material, of the varieties best adapted to the specifics of the culture area, is a valuable way to implement these strategies.

MATERIALS AND METHODS

The observations and determinations were carried out in the wine-growing center Banu Mărăcine, a wine-growing center located in the demarcated area for the production of wines with the Controlled Designation of Origin (DOC) "Banu Mărăcine", in a 7 year old vineyard, in the Fetească neagră variety grafted on Teleki 4 the selection Oppenheim 4 rootstock (SO4), with 2 x 1.2 m planting distance, semi-high growth, Single cordon training system, 12 bud/m^2, without irrigation.
In accordance with the research topic proposed, the observations and determinations focused on: monitoring climatic factors for the evaluation of the favourableness of the study year (2021) and studying the influence of the climatic conditions characteristic to the study years on the physiological and bioproductive parameters. For the evaluation of the climatic conditions, the meteorological data from the weather station located in the Banu Maracine wine center as well as the data provided by WorldWeatherOnline were used.
The effect of the varied climatic resources was evaluated through the analysis of the physiological indicators, and bioproductive parameters: evolution of weight, volume, acidity and sugar content of berries during ripening.
The determination of the physiological indicators (the intensity of transpiration and photosynthesis, stomatal conductance) was made with Lci-pro equipment during the vegetation period, in the second decade of each month.
In order to highlight the effect of the hot temperatures on stomatal conductance and transpiration the determinations were made in two days with different thermal profile (day with normal temperature and day with hot temperature, > 40^{0}C)

RESULTS AND DISCUSSIONS

For a better appreciation of the value of the main climatic determinants from the experiment period, they are presented together with the multiannual average values for the period 2010-2022 (Figures 1-6)
Comparing the average monthly temperatures recorded during the experiment period (Figure 1) with the multi-year average (Figure2), higher values of the temperatures are found, especially in the period from June to September, months in which the average temperature values for the year 2021 were about 2 degrees Celsius higher, values which determined changes in the manifestation of the studied physiological processes.

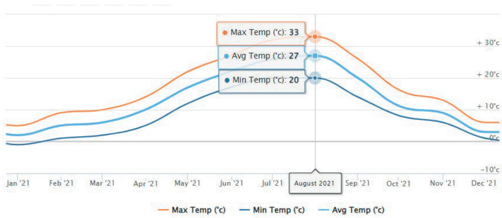

Figure 1. Monthly maximum, average and minimum temperatures during the experimentation period

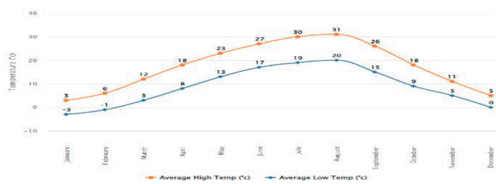

Figure 2. Average temperature (2010-2022)

The recorded values of insolation indicate the favourability of the crop year for vines, with higher insolation values being registered between July and September, a fact that influenced the determined physiological and bioproductive indices (Figure 3).

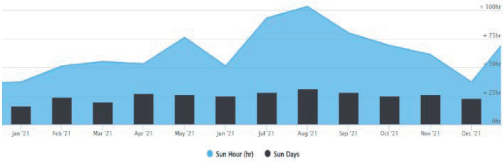

Figure 3. Sun hours and Sun days during the experiment period

Regarding insolation, no significant differences were recorded during the experiment period compared to the multiannual average.

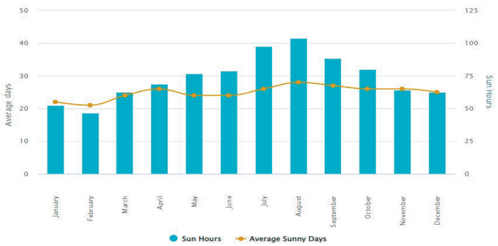

Figure 4. Average Sun hours and Sunny days (2010-2022)

Studying the data on precipitation during the experimentation period (Figure 5) a larger amount is found in the first part of the vegetation, period April-June, and the lack of useful precipitation (over 10 mm) during the ripening period (August-September).

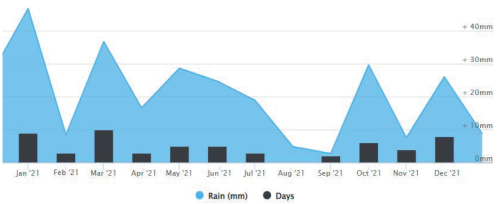

Figure 5. Rainfall amount and rainy days during the experimentation period

Lower precipitation values for the year 2021 are also found compared to the multi-year average (2010-2022) for the entire duration of the vegetation period - Figure 6.

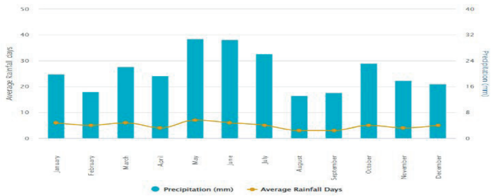

Figure 6. Average rainfall monthly amount and rainy days (2010-2021)

The determinations of the physiological indicators carried out dynamically, during the vegetation period, show the increase in the intensity of the physiological processes (photosynthesis and transpiration) until July when they reach values of 7.5 µ mol/m^2/s respectively 3.9 mol/m^2/s - Figure 7. Starting with August as a result of the specific climatic conditions of the year (lack of precipitation and temperatures above the multiannual average, the intensity of transpiration is reduced more compared to the intensity of photosynthesis.

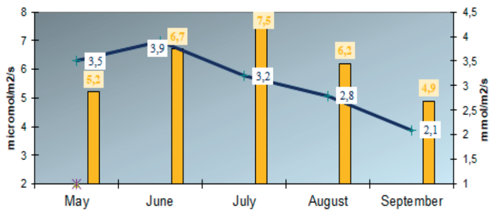

Figure 7. The the intensity of photosynthesis and transpiration in monthly dynamics throughout the vegetation period

Starting with August as a result of the specific climatic conditions of the year (lack of precipitation and temperatures above the multiannual average, the intensity of transpiration is reduced more compared to the photosynthesis rate which remains at around 6.2 respectively 4.9 µ mol/m^2/s - Figure 7.

The determinations made during a day with a climate profile specific to the heat wave, with temperatures over 40^{0}C, showed the correlation that existed between the intensity of transpiration and the stomatal conductance. On 29.07.2021, when the determinations made revealed temperatures at the leaf level of almost 44^{0}C, it was possible to determine, at the same time, the variation in the values of transpiration and stomatal conductance (Figure 8).

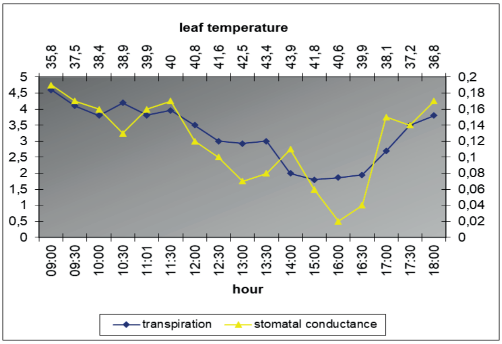

Figure 8. Transpiration intensity and stomatal conductance in day with on a day with hot temperatures

From the determinations made, we notice that the stomatal conductance has a more pronounced decrease in values compared to transpiration. At the same time, it can be observed that the 2 indices do not always keep the same trend, there are moments when the stomatal conductance values have more intense increases (Figure 8). The closing of the stomata is an adaptation mechanism that prevents dehydration, reducing sweating. They do not close completely during the entire duration of high temperatures, there are moments of higher intensity of gas exchange that alternate with moments of lower intensity. This fact also allows transpiration with less intensity but can contribute to regulating the temperature of plants affected by hot temperatures.

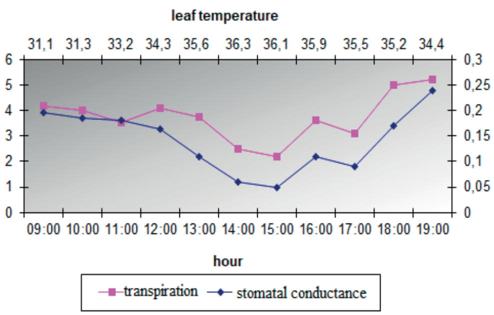

Figure 9. Transpiration intensity and stomatal conductance in day with normal thermal profile

The determinations made on a day with normal thermal profile (22.07.2021) highlights stomatal conductance transpiration and transpiration and transpiration and transpiration values on a day with a normal thermal profile in which the values of the two physiological indices are not affected by excessive temperatures (Figure 9). At temperatures that do not reach restrictive values, the stomata allow gas exchange and transpiration with normal intensities depending on the other environmental factors.

As a result of interaction with the variable climatic conditions, the vine plants react by regulating the physiological and biochemical processes that result in quantitative and qualitative changes at the level of the whole plant and especially of the grape.

The analysis of the determinations made on the Fetească neagra variety regarding the bioproductive indicators that are the subject of this study, highlights the dependence of reaching the moment of maturity on the environmental conditions, which determine a different rate of growth of the berries, of the accumulation of substances that determine the quantity and quality of production. Figure 10 shows the dynamic evolution of berry weight and volume during ripening (from the beginning of berry colouring to harvest).

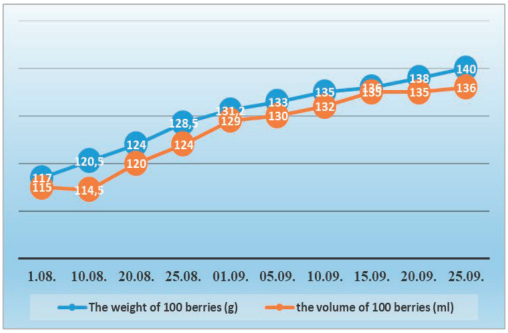

Figure 10. The dynamic evolution of the weight and volume of the berries during ripening

Analysing figures 10 and 11, we can see that the very small amounts of precipitation from July to September had a greater influence on the quantitative characteristics of the grapes, with the grapes having smaller dimensions and volume than normal (Figure 10). As for the quality indices, they were not negatively influenced (Figure 11). This fact could be explained by the existence of a quantity of accessible water in the soil, due to the precipitation from the first phases of vegetation. It can be noted that in 2021, about 80% of the total precipitation was recorded in the first phenological stages.

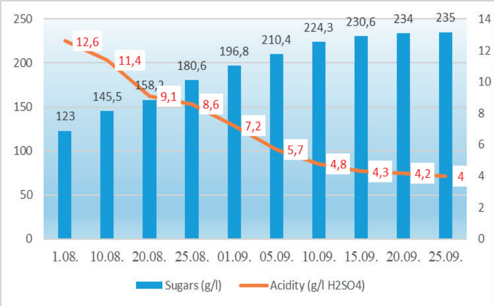

Figure 11. Evolution of sugar content and acidity of berries during maturation

Argument in this regard is the carbohydrate content recorded on September 25 - 235 g/l, under the conditions of an acidity of 4 g/l H2SO4 - Figure 11.

The Fetească neagră variety taken in the study demonstrated a high accumulation potential, the sugar parameter increasing by 113 g/l in the period 1.08.-25.09. under the conditions of a final acidity of 4 g/l.

In the climate context of year 2021, insolation (including the heat component) had the highest rate of participation in the development of physiological reactions generating a high level of quality, a result also influenced by the reduced amount of precipitation in the second part of the vegetation period.

CONCLUSIONS

The climate has a determining role on the growth and development of the vine. Among the most common effects of the variation of climatic conditions on the vine are those related to the change of phenology and the duration of the vegetation period, influencing the intensity of physiological processes and metabolism, and finally determining quality and quantity of production. Knowing and modelling the action of these changes can be a key tool for planning viticultural management practices under conditions of a climate characterised by a high level of variability.

The diversity of climatic conditions during the research period was reflected in the variations of the studied physiological and bioproductive indicators.

The Fetească neagră variety has interacted with environmental conditions, expressing its adaptive potential in a specific way.

In the conditions of the year 2021, as a result of the precipitation that fell at the beginning of the vegetation period, the studied variety faced the dry temperature wave, without major impact.

Under the conditions of temperatures over 40^0C, the stomatal conductance was significantly reduced, but the stomata continued to open, allowing transpiration to occur with a lower intensity. This fact contributed to maintaining the temperature of the plants during the respective period.

In conclusion, the 2021 vintage did not meet the conditions for a high level of production, but it can be considered a high-quality production year.

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THE INFLUENCE OF THE CLIMATE ON THE EVOLUTION OF DISEASES IN THE SAUVIGNON BLANC VARIETY IN THE CONDITIONS OF THE YEARS 2021-2022 IN MURFATLAR

Ionica DINA[1], Sergiu-Ayar ENE[1], Victoria ARTEM[1], Aurora RANCA[1], Mihaela HNATIUC[2]

[1]Research Station For Viticulture And Enology Murfatlar, Calea București 2, Murfatlar, Romania
[2]Maritime University of Constanta, Mircea cel Bătrân 104, Constanța, Romania

Corresponding author email: ayar_ene@yahoo.com

Abstract

The years 2021 and 2022 evolved differently from a climatic point of view, one being particularly rainy and the other being relatively dry. During this period, through the ERA-Net MERIAVINO project, studies were carried out on the Sauvignon blanc variety treated and untreated variants with phytosanitary protection substances regarding the degree of attack on the main diseases - Downy Mildew, Powdery Mildew and Gray Rot, along with determinations regarding stomatal conductance and sensor recordings for leaf humidity. The determinations that were made showed that the climatic factors, especially the precipitation and the relative humidity of the air have an uneven distribution during the vegetation period; determine the occurrence of cryptogamic diseases and have a great influence over the rate of extension of grapevine diseases (GA). In 2021, the degree of pathogen attack was higher compared to 2022, being directly correlated with the value of leaf humidity and also, the stomatal conductance determined for 4 days immediately after the appearance of the first symptoms of Plasmopara viticola and Botrytis cinerea infections recorded decreasing values.

Key words: *diseases, grapevine, leaf humidity, stomatal conductance, untreated.*

INTRODUCTION

The wine production health status is strongly influenced by the annual evolution of environmental factors. The abiotic factors are represented by the climate, and the biotic ones by the development of cryptogamic diseases and pests.

Observations carried out over the years, regarding the appearance, spread and evolution in time and space of the main phytopathogenic agents of the grapevine: grapevine Downy Mildew (*Plasmopara viticola*, Berk. & M.A. Curtis - Berl. & De Toni), Powdery Mildew (*Uncinula necator*, Schwein) and Gray Rot (*Botrytis cinerea* Pers.) revealed different levels of attack, depending on the climatic conditions of each year (Bois et al., 2017; Caffarra et al., 2012), and the presence of the inoculum, disease history and variety sensitivity (Tomoioaga, 2013).

Phytopathogens strongly act on the plant at the biochemical, physiological and anatomical-morphological levels (Moriondo et al., 2005). The stomata constitute areas of penetration to the leaf tissues for numerous pathogens such as bacteria, fungi such as *Plasmopara viticola*. Many pathogens induce the closing of the stomata, and in some cases induce their abnormal opening and accentuate the loss of water from the plant (Duniway et al., 1971).

Monitoring the evolution of pathogens, time and development phases plays an important role in the detection and optimal treatment of diseases and has an important impact on the improvement of production and its quality. Pathological processes lead to physiological changes and affect the amount of biomass produced by each individual plant (Rabbinge et al., 1989). The decrease in biomass accumulation in plants affected by pathogens is caused by the appearance of necrotic points on the leaves, which leads to a decrease in the surface of the leaf apparatus and the interception of photons. Necrotic spots are associated with decreased photosynthetic efficiency in dead leaf tissues, but also in adjacent or intact areas of the leaf, leading to decreased CO_2 assimilation in affected tissues and leaving CO_2 assimilation intact in the rest of the leaf surface with healthy tissue.

To ensure a sustainable agriculture, it is necessary to monitor the health of plants to limit the spread of diseases and to reduce the degree of production damage (Van Oijen, 1990).

MATERIALS AND METHODS

The study was carried out in 2021 and 2022 on the Sauvignon blanc variety from the vineyard plantations at the MURFATLAR Research Station for Viticulture and Oenology. The plantation cultivated with the Sauvignon blanc variety grafted on Berlandieri x Riparia Sel rootstocks. Opp. 4-4 was established in 2011, having a slope distance of 2.2/1.1 m, semi-tall Guyot driving form with 70 cm stem, bilateral cord..

The soil maintenance system kept the soil bare. The experimental lot had an untreated and a treated variant.

In the untreated experimental variants, in order to stimulate the infection with *Plasmopara viticola, Uncinula necator* and *Botrytis cinerea*, optimal conditions were created: the leaf apparatus was moistened periodically and permanent surfaces with water were created under the selected trunk, to stimulate the spread of *Plasmopara viticola* and *Botrytis cinerea*, and in the treated areas phytosanitary treatments for prevention and control were carried out year after year.

To monitor the climatic elements, the weather station (iMetos 3.3), located in the center of the vineyard, was used. To identify the vegetation phenophases of the grapevine, the BBCH Lorenz Scale (Lorenz et al., 1995) was used, and to determine the frequency (F %), intensity (I %) and degree of attack (G.A. %) the visual graphic scale described by Buffara was used for the leaves (Buffara et al., 2014), based on seven levels of disease severity: 1, 3, 6, 12, 25, 50, and 75%, and for clusters, the scale diagram with the seven levels of disease severity: 5, 15, 25 , 35, 50, 75 and 100% - Caffi (Caffi et al., 2010).

Monitoring of pathogen evolution, time and development phases was carried out twice a week.

During the observations, in 2022, the stomatal conductance was determined with the "Steady State" porometer over leaves infected with *Plasmopara viticola* and *Botrytis cinerea.*

For Downy mildew (*Plasmopara viticola*), measurements were made for 4 days during the incubation period (starting with BBCH 11, and 3-4 days from the moment the first spots appeared on the vine leaves (BBCH 11-19), and for Gray Rot (*Botrytis cinerea*), the measurements were made from the moment the first spots appeared on the clusters (BBCH 69-89).

RESULTS AND DISCUSSIONS

Climatic data

The climatic data of the years studied showed a large variation in the precipitation regime and an uneven distribution during the vegetation period, which caused pathogens to appear in all the years of the study, manifesting different levels of attack on the grapevine, depending on the climatic conditions and the vegetation phenophases.

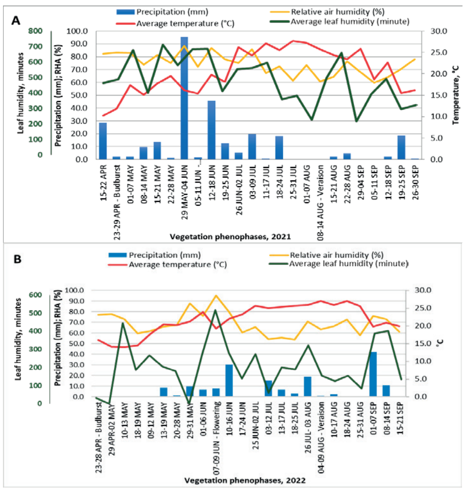

Figure 1. Climatic data and the main phenological stages of the grapevine, registered during the study period from April to September: in A, 2021 and in B, 2022

In 2021, during the growing season, precipitation totaled 339.3 mm and in 2022 they summed 206.7 mm.

As can be seen in Figure 1 A and B, the rainfalls recorded in the months of May and June created favorable conditions for the outbreak of the Downy Mildew (*Plasmopara viticola*) starting from May every year. The very high temperatures in the months of July and August of 2021 and the short intervals with atmospheric drought, the low humidity at the leaf level and the slightly increased temperatures above 25°C in the months of June, August and September of the year 2022 favored the late appearance of Powdery Mildew (*Uncinula necator*). Regarding the manifestation of Gray Rot (*Botrytis cinerea*) in 2021, it started with the beginning of veraison and in 2022, it set in after the downpour accompanied by hail on 14.06.2022, and evolved and amplified in intensity in the following months.

The evolution of diseases on phenophases of vegetation

In 2021, Downy Mildew was triggered in the untreated variant on 17.05.2021 (BBCH 15) and on 04.06.2021 in the treated variant and in 2022 the first Downy Mildew spots were identified on 14.06.2022 at both variants (untreated and treated).

Due to the large amount of precipitation recorded in the months of May, June and July, which maintained a hygroscopicity between 70 and 88% and a leaf humidity level over an average interval of 228-711 minutes, in the year 2021, the degree of attack in the plantation of the disease caused by the phytopathogenic agent *Plasmopara viticola* had a progressive increase in the untreated variant starting with the onset of flowering (BBCH 60) - GA = 6.46% and recorded a sudden increase when the berries stood out well within the cluster (BBCH 73), reaching a value of 27.24%. In the treated variant, the degree of attack on the leaves reached the value of 12.32% (BBCH 73) (Figure 2).

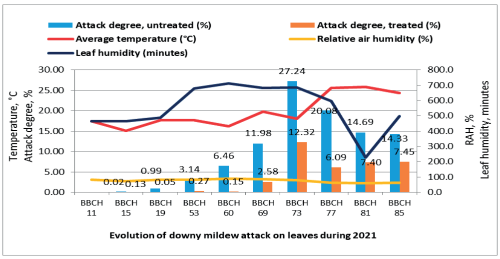

Figure 2 - The evolution of the Downy Mildew (*Plasmopara viticola*) on leaves in 2021

The degree of attack on grapes followed the same evolution, registering the highest point in the BBCH 73 phenophase. It had a value of 31.6% (Figure 3).

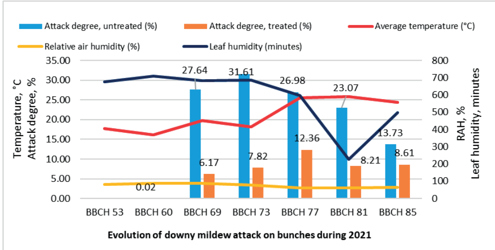

Figure 3. Evolution of Downy Mildew (*Plasmopara viticola*) on grapes in 2021

In 2022, the frequency and intensity of the attack produced by *Plasmopara viticola* on the leaf apparatus was very reduced, on the other hand, on grapes, in the untreated variant, the degree of attack recorded a percentage of 1.32% and 1.56% respectively (BBCH 69 and BBCH 73), this being determined by the rain shower accompanied by hail from 14.06.2022, by a hygroscopicity of 95.2% and an average leaf humidity that persisted for an average interval of 517 minutes (Figure 4).

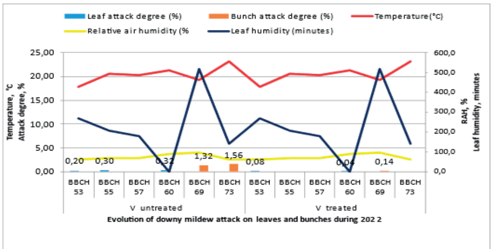

Figure 4. The evolution of Downy Mildew (*Plasmopara viticola*) on leaves and grapes in 2022

The Powdery Mildew (*Uncinula necator*) in the Murfatlar viticultural center manifests itself with a very high intensity due to the climatic conditions in the area very favorable for the appearance of the pathogen (very high air temperatures, very low atmospheric humidity). The first outbreaks of infection produced by *Uncinula necator* corresponded to the phenophase of growth and development of berries (BBCH 73) and was identified in the untreated variant on 22.06 2021. The degree of attack on the leaves was lower. On the other hand, there was an progressive evolution on the grapes at the beginning of the ripening of the berries (BBCH 81) - a degree of 4.99%.

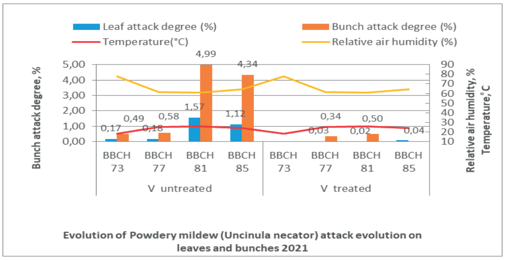

Figure 5. The evolution of the Powdery Mildew (*Uncinula necator*) on leaves and grapes in 2021

In the treated variant, the infection started when the clusters were compacted - BBCH 77 on 22.07.2021 and registered a higher degree of attack at the start of veraison (BBCH 85) - 0.50% (late infection), (Figure 5).

In 2022, the maximum degree of attack recorded on leaves was 0.30% (BBCH 53) and on grapes it reached 3.34% in the BBCH 73 phenophase (Figure 6).

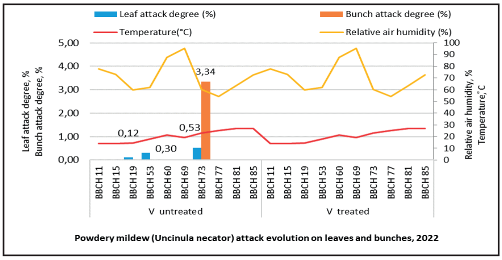

Figure 6. The evolution of the Powdery Mildew (*Uncinula necator*) on leaves and grapes in 2022

In Murfatlar, the Gray Rot (*Botrytis cinerea*), causes significant damages in rainy years, during the veraison and ripening period, and in dry periods it ennobles the quality of the harvest (Noble Rot). Both years were favorable for the development of the biological cycle of the Gray Rot (*Botrytis cinerea*) due to rainfalls, the relative air humidity and the high temperature at full maturity. In the year 2021, in the untreated variant, the infection with *Botrytis cinerea* was triggered, at the beginning of veraison (BBCH 81) and had a high degree of attack 27.64% in the grape maturity phenophase (BBCH 89). In the treated variant registered at full maturity BBCH 89, a percentage of 6.26% (Figure 7).

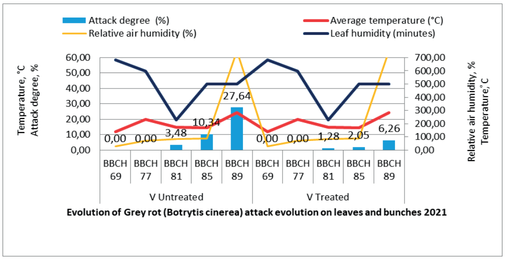

Figure 7. Evolution of the attack of gray rot (*Botrytis cinerea*) in 2021

In 2022, following the downpour accompanied by hail on 14.06.2022, which produced slight cuts on grapes, the infection with *Botrytis cinerea* was triggered, which evolved and increased in intensity in the following months, registering a high degree of attack - 14.32% in the untreated variant and was kept below 1.88% in the treated variant by applying specific phytosanitary treatments for Gray Rot (Figure 8).

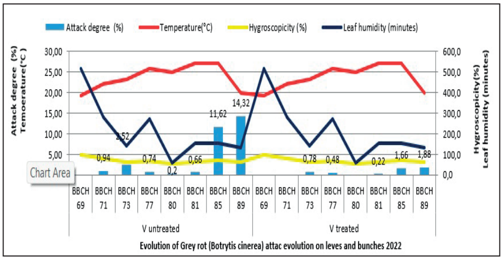

Figure 8. Attack evolution of Gray Rot (*Botrytis cinerea*) in 2022

Stomatal conductance

In 2022, during 4 consecutive days from the appearance of the first symptoms of infection with *Plasmopara viticola* (Figure 9), the stomatal conductance in the treated and untreated variants had a tendency to decrease, which means that the exchanges of gases between the plant and the environment are reduced with the increase in the intensity of the pathogen's attack. In the untreated variant, from values that reached 189.9 mmol/m^{2}s^{-1} on the first day, it decreased to 114.15 mmol/m^{2}s^{-1} on the last day of measurements, and in the treated variant, from 73.9 mmol/m^{2}s^{-1}, on the first day, to 55.48 mmol/m^{2}s^{-1} on the last day. The values determined in the untreated version were higher compared to those in the treated variant.

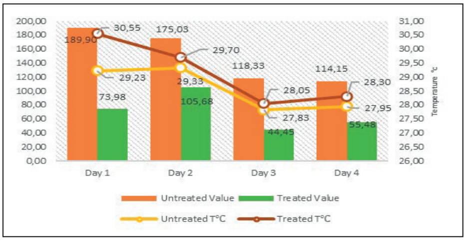

Figure 9. The evolution of the stomatal conductance measured in 2022, in the first 4 days after the appearance of the first symptoms produced by *Plasmopara viticola*

With the appearance of the first lesions on clusters, following the onset of the Gray Rot attack (*Botrytis cinerea*), in the 4 consecutive days of determinations, the stomatal conductance recorded decreasing values from 48.55 $mmol/m^2s^{-1}$ on the first day to 11.59 $mmol/m^2s^{-1}$ on the last day of observation (Figure 10).

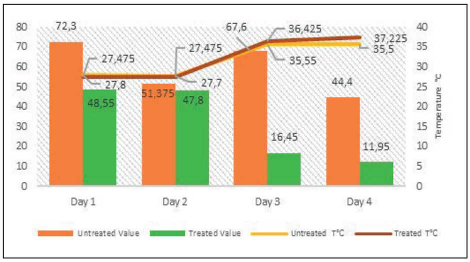

Figure 10. The evolution of the stomatal conductance measured in 2022, the first days after the onset of infection with *Botrytis cinerea*

CONCLUSIONS

The thermal oscillations and the uneven distribution of rainfalls during the growing season, relative air humidity and the persistence interval of leaf humidity, determined the occurrence of cryptogamic diseases and greatly influenced the rate of extension of vine diseases (GA). The aggressiveness of the diseases was higher in 2021 compared to 2022, and gas exchanges (stomatal conductance) recorded decreasing values in the first 4 days immediately after the appearance of the first symptoms of *Plasmopara viticola* and *Botrytis cinerea* infections. The correct development and application of a disease protection program cannot be achieved without the correct diagnosis, which requires specialized personnel, time and a large number of observations in the field. In this case, the accurate identification in the shortest possible time before the establishment of infections produced by pathogenic agents is an essential condition for the application of timely and economically effective treatment prevention measures.

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INTEGRATED MANAGEMENT OF VINEYARD PLANTATION - COMPARATIVE STUDY OF OLIVIA VARIETY AND FETEASCA NEAGRA 4VI CLONE

Oana-Silvia DINA VLĂDUCA[1], Roxana HOROIAȘ[2], Marian ION[3], Cezarina NECULA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania, Mobile: +40733520539, Emails: oana.dina.vladucu@gmail.com, inanecula2004@yahoo.com
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Management, Economic Engineering in Agriculture and Rural Development, Slatina Branch, 150 Strehareti Street, 230088, Slatina, Olt, Email: roxana.horoias@gmail.com
[3]ICDVV Valea Călugărească, 2 Valea Mantei Street, Valea Călugărească, 107620, Prahova County, Romania, Email: marian1367@yahoo.com

Corresponding author email: oana.dina.vladucu@gmail.com

Abstract

Knowledge of the biological, ecological, technological and managerial peculiarities applied in vineyard, as well as their integration in the context of sustainable development and the economic and resource crisis, is a permanent concern of the specialists working in this field of activity. This paper presents some of the results obtained from the research carried out within the ICDVV Valea Calugareasca during three agricultural years (2017-2019) regarding the management of the vineyard. The behaviour and adaptation to climate change of the Olivia variety and the Feteasca neagra 4VI clone were observed. These were chosen due to the fact that both were obtained at ICDVV Valea Calugareasca, they are intended for winemaking, they have the same ripening period (IV-V, depending on the characteristics of the agricultural year), and their production potential is similar (9.3 t/ha in the variety and 10.0 t/ha in the clone). The results indicate that, under identical eco-pedological and technological conditions, the Feteasca neagra 4VI clone adapted better to the climatic conditions of the research years, managing to obtain 9-16% higher yields compared to the Olivia variety.

Key words*: vineyard management, wine grapes, variety, clone, yields.*

INTRODUCTION

In recent years, more and more winegrowers and researchers in the field have come to the conclusion that the selection of clones is a gain for viticulture, both from the point of view of high productions and their quality (Alecu et al., 2018; Burin et al., 2011; Costescu et al., 2010), as well as of the favorable response to the tested agro-technologies (Bigot et al., 2022).

Recently, the management of the vineyard, as well as the improvement activity through the selection of valuable clones, also consider adaptability to climatic conditions (Bucur, 2021; Ranca et al., 2022; van Leeuwen & Destrac-Irvine, 2017), which variability is constantly increasing, influencing the good development of the plants and, likewise, the annual yields.

Grape genotypes for wine are more and more exposed as they have to meet the specific requirements of the respective variety (Tudorache et al., 2014), with particular reference to the amount of accumulated sugar and acidity (Dimovska et al., 2013) - both directly influenced by abiotic factors. As the vineyards are located in arid areas, where rainfall decreases from one year to the next, varieties and clones are needed to use the available water as efficiently as possible (Mairata et al., 2022).

The value of clones has been confirmed by practice over more than a century, observing that they remain constant as long as no further mutation occurs, and then a new selection is required (Hajdu et al., 2011), which correspond to the new demands of the environment.

It should also be mentioned that, depending on the variety and clone, both in the case of table grapes and those for wine, the agrobiological and technological characteristics differ (Przic & Markovic, 2019), therefore it’s necessary to constantly carry out research and to monitor very carefully any changes that appear (Salimov et al., 2022). Research institutes for viticulture and winemaking all over the world started the selection of clones from native varieties (Filip et al., 2018; Sivcev et al., 2011), which they tested by replacing old cuttings, following their evolution, establishing their characteristics and homologating the most valuable ones.

Considering all the above, we aimed to test to what extent a variety and a clone with similar characteristics adapts to the new climatic conditions of the area where the test field is located. The climatic conditions (temperature and precipitation) for the years 2017-2019 were followed, and depending on them the entry into the main phenophases and the obtained yields.

MATERIALS AND METHODS

In a period of three years (2017-2019), at the Research Institute for Viticulture and Winemaking in Valea Calugareasca (ICDVV), were carried out research that aimed to monitor the main climate elements for the period of maximum interest for the vineyard, namely the months of April-October, which were later correlated with the quantitative and qualitative results of each year's yields.

Most of the vineyards of the ICDVV are found at altitudes between 110 and 260 m. Reddish-brown soils with a heavy texture, loam-clay and clay-loam predominate, with a humus content in the range of 1-3.3%. The apparent density of the soil shows, in the bare horizon (0-60 cm) of most soils, average values (1.20-1.35 g/cm^3), but also high values (1.35- 1.48 g/cm^3), depending on the texture and the state of loosening or settling of the soil.

The physical-chemical composition of the soils, both in terms of pH, the degree of saturation in bases and the presence in moderate proportions of $CaCO_3$ ensure a good quality of yield, especially of the varieties intended for high-quality red wines.

The local climate of the area where the Valea Calugareasca viticultural center is located is temperate continental, with early springs, hot summers, mild and prolonged autumns and less harsh winters, without excluding the frosty periods that may occur in certain years. The data recorded at the Valea Calugareasca weather station (latitude 44°59', longitude 26°13', altitude 210 m) were used to prepare the study on the climate of the wine-growing location.

As plant material, the Olivia grape variety and the Fetească neagră 4VI clone were used, both of which are ICDVV's own creation, intended for winemaking and having the comparative characteristics presented in Table 1.

Table 1. Properties of the Olivia variety and Fetească neagră 4VI clone - a comparative look

Crt.	Index \ Genotype	Olivia variety	Fetească neagră 4VI clone
1.	Homologation year	2003	2010
2.	Parents	(Babeasca neagra x Pinot noir) x Pinot noir	Feteasca neagra population
3.	Ripening date	IV-V (10-30.09)	IV-V (10-30.09)
4.	Average grape weight (g)	111	199
5.	Average grain weight (g)	1.2	1.8
6.	Sugar in must (g/l)	202	194
7.	Must acidity (g/l H_2SO_4)	4.5	3.8
8.	Yield potential (t/ha)	9.3	10.0

The plantation where the research was carried out was established in 2011, in order to follow the evolution of the Olivia variety and the Cabernet Sauvignon 30VI and Feteasca neagra 4VI clones in parallel. The Cabernet Sauvignon 30VI clone wasn't included in the study, mainly due to differences in ripening age and productive capacity.

So, benefiting from identical pedo-climatic and management conditions, the behaviour of the variety and the clone was followed in terms of the elements of fertility and productivity, respectively:

- RFC - relative fertility coefficient;
- AFC - absolute fertility coefficient;
- RPI - relative productivity index;
- API - absolute productivity index.

The results obtained after the analysis of the six plots monitored annually, three from the Olivia variety, and three from the Fetească neagră 4VI clone, were managed in tables, and later processed statistically with the help of Anova and Table Curve 3D programs. The aim of the research was to compare how the variety and the clone reacted to the climatic conditions of the three years of study, so that it could be concluded whether or not the clone brings an increase in viticulture, in the case of applying an integrated management, similar to the one used until now for wine grape varieties.

RESULTS AND DISCUSSIONS

Compared to the values taken as a reference, namely the average of the years 1985-2016, the main indicators related to temperatures, precipitation and hygroscopicity were tracked (Table 2), all of which contribute essentially to the quantity and quality of grapes obtained at the end of a production cycle. The average monthly temperatures from April-October 2017-2019 were, on average, very close, even identical to the multi-year ones, and analyzing them monthly, some differences of 1-2°C are observed, but they are adjusted by lowering the average in one of the between previous or following months. With a few exceptions (the month of September 2019, with only 3.8 mm of precipitation), it can be seen that no extremes were recorded during the analyzed period. In addition, in the months of maximum importance for the formation of production elements, the amount of precipitation exceeded that of the control in each of the three years, so that the crop didn't suffer from lack of water.

Table 2. The thermal regime in the months of April-October of the years 2017, 2018 and 2019, in the studied area

Month	Monthly average temperature (°C)				Precipitations (mm)				Hygroscopicity (%)			
	Normal	2017	2018	2019	Normal	2017	2018	2019	Normal	2017	2018	2019
IV	11.7	10.9	14.7	11.2	44.8	107.0	65.0	74.8	67.7	68.3	67.0	74.8
V	17.5	18.4	15.6	17.0	67.3	56.4	82.2	190.6	68.4	65.9	75.0	77.6
VI	21.5	21.2	22.1	23.6	81.5	84.7	81.8	85.6	70.1	69.5	72.3	70.9
VII	23.6	22.9	23.7	22.7	75.8	86.6	70.2	68.4	67.5	71.0	63.3	44.6
VIII	23.3	24.1	22.7	24.2	62.7	36.4	78.2	37.0	66.5	61.1	65.4	60.9
IX	18.1	19.4	19.0	19.1	54.4	40.2	66.0	3.8	70.7	65.3	65.1	57.9
X	12.2	10.5	12.1	12.5	46.2	86.8	120.0	15.6	77.0	76.4	82.0	69.6
Mean/Sum	18.27	18.20	18.56	18.61	432.7	498.1	563.4	475.8	69.7	68.2	70.0	65.2

Source: own data, measured at the Valea Calugareasca meteorological station

Air hygroscopicity (relative humidity), an indicator of maximum importance for vines, had average values, close to those of the control period. There was also an exception here, namely the month of July 2019, when it dropped to 44.6%, without representing a critical situation and which didn't perpetuate itself in the following months. At the same time, it is observed that during the period of grain growth, when the requirement is 70-80% hygroscopicity, these values weren't reached, so the maximum yield potential of the variety and/or clone couldn't be reached either, through the lens of this parameter.

Depending on the clues presented, the times at which the variety and clone phenophases were reached in each of the three years can be explained (Table 3).

Table 3. The dates of reaching the phenophases by the Olivia variety and the Feteasca neagra 4VI clone, in 2017-2019

Phenophases	Bud burst			Flowering			Verasion			Harvest		
Year / Genotype	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Olivia	11.04	16.04	23.04	17.06	9.06	18.06	13.08	7.08	16.08	12.09	18.09	7.09
Feteasca neagra 4Vl	15.04	18.04	12.04	13.06	19.06	10.06	15.08	18.08	12.08	10.09	25.09	13.09

Source: own observations, from the study location from Valea Calugareasca

In 2019, in the Olivia variety, ripening occurs early, three days before the start of the period. Outside of this particular situation, ripening occurs within the optimal range. It should also be noted that, within the same year, several of the phenophases have different periods for the variety and the clone, that is why in 2017, for example, the Olivia variety blooms first, but the Feteasca neagra 4VI clone blooms first, enters the fall earlier the Olivia variety, but the clone reaches maturity faster. Such successions also occur between years, there being no constant in terms of phenophases.

To calculate the elements of fertility and productivity, the classic calculation formulas were used, and in order to obtain the RFC, AFC, RPI and API values, 10 grape vines, respectively 10 bunches from each research batch were analyzed, in order to establish:

- the total number of shoots per vine;
- the number of fertile shoots per vine;
- the number of inflorescences;
- average weight/grape (g).

Yield (t/ha) was calculated by finding the average yield/vine, which value was then multiplied by the number of vines/ha. The average of the results for each of the three research years is centralized in Table 4, and the values of the fertility and productivity elements were processed with Anova, resulting in the graphs in Figure 1.

Table 4 shows that the percentage of fertile shoots (FS) is, without exception, higher in the Olivia variety, with values between 76 and 81%. On the other hand, the Feteasca neagra 4VI clone, which anyway we observe that normally has a lower total number of shoots per vine, also has a lower yield of fertile shoots, with FS between 62 and 71%.

The proportions are reversed when we analyze another parameter, the average weight of the grapes (g), which is favorable to the Feteasca neagra 4VI clone - compared to the Olivia variety there is an increase of 20-25%, compensating for the lower number of shoots/vines, as well as that of inflorescences and fertile shoots.

Table 4. Yields parameters for the Olivia variety and Feteasca neagra 4VI clone, measurements from the research period, 2017-2019

Year	Genotype	Total no. of shoots per vine	No. of fertile shoots (FS) per vine	No. of inflores-cences	RFC	AFC	FS (%)	Average weight/grape (g)	RPI	API	Yields (t/ha)
2017	Olivia	46.2	36.8	45.3	0.98	1.24	79.94	112.14	109.89	139.05	7.81
	Feteasca neagra 4 VI	33.9^{OOO}	24.4^{OOO}	33.1^{OOO}	0.98	1.37	71.80	133.79	131.11	183.29	9.05
2018	Olivia	32	26	36	1.13	1.38	81.30	111.78	126.31	154.25	8.10
	Feteasca neagra 4 VI	32	20^{OO}	27^{OOO}	0.84	1.35	62.50	130.97	110.01	176.81	8.98
2019	Olivia	49.4	37.7	51.7	1.06	1.38	76.40	105.72	112.06	145.89	8.33
	Feteasca neagra 4 VI	32.6^{OOO}	23.1^{OOO}	35.4^{OOO}	1.07	1.50	70.90	130.77	139.92	196.16	9.50

Source: own data, observations and calculations.

Coming back to the fertility and productivity elements, they were calculated using the following formulas:

$$RFC = \frac{No.\ inflorescences/vine}{Total\ no.\ of\ shoots/vine} \quad (1)$$

$$AFC = \frac{No.\ inflorescences/vine}{No.\ fertile\ shoots/vine} \quad (2)$$

$$RPI = RFC \times Average\ weight/grape\ (g) \quad (3)$$

$$API = AFC \times Average\ weight/grape\ (g) \quad (4)$$

Being an analysis with two genotypes, over a period of 3 years, with four elements of fertility and productivity, the trifactorial analysis performed with the statistical program Anova resulted in obtaining four different graphs, one for each coefficient (RFC and AFC) and indicator (RPI and API). The 4 graphs, presented in Figure 1, have been numbered with letters (a-d) and they highlight the fact that the Olivia variety is equal or superior to the Feteasca neagra 4VI clone only in terms of the relative fertility coefficient (RFC), and here the differences are smaller or higher, depending on the particularities of the year.

Close values are found for the absolute fertility (AFC), which is always above unity, falling into the middle fertility category.

The yield, expressed in t/ha, was analyzed by entering the data into the Table Curve 3D statistical analysis program, with the help of which we obtained a graph (Figure 2) from which we can see the differences between the Olivia variety and the Feteasca neagra 4VI clone. It should also be mentioned here that the yield was continuously increasing, this is due to both biotic and abiotic factors, as well as the maturity of the crops, established in 2011, now starting to approach the era of maximum productivity and of reaching the potential of the genotype.

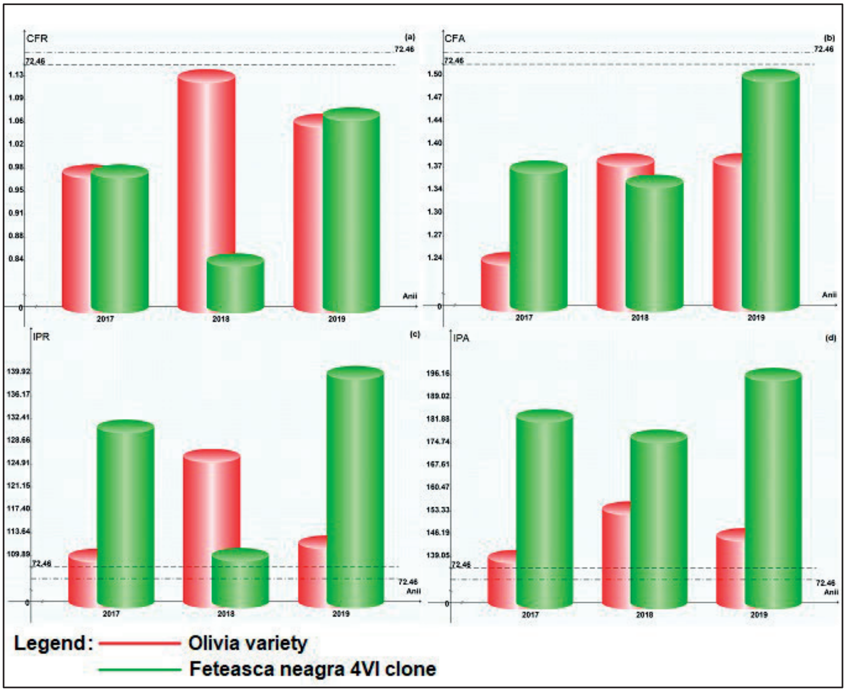

Figure 1. Graphs highlighting the differences between the Olivia variety and the Feteasca neagra 4VI clone, in terms of RFC (a), AFC (b), RPI (c) and API (d), in the years 2017-2019
(Source: own determinations and analysis)

The differences between the variety and the clone are maintained throughout the monitoring years (2017-2019), being between 9 and 16%, each time in favor of the clone. Taking into account all the other parameters that led to finding out the production, we can say that the Olivia variety is limited, in the formation of production elements, by the weight of the grapes (g), which otherwise limits its yield.

On the other hand, Feteasca neagra 4VI clone could have a much better yield if it would have managed to have a higher number of fertile shoots per vine, a category in which it loses a lot. It can therefore be stated that each of the analyzed genotypes has pluses and minuses, but also that both are productive and don't require a particular management, managing to obtain competitive results with classic technologies, already consecrated, to which they respond very well.

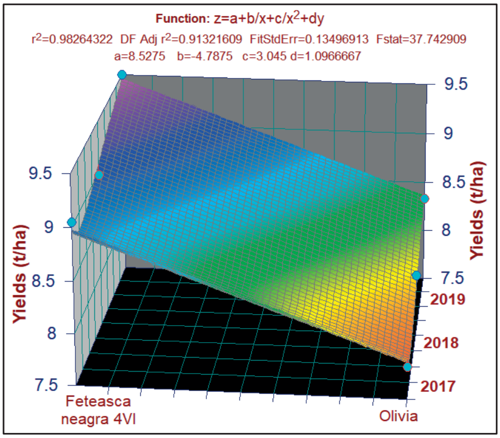

Figure 2. Three-dimensional graph of the productions made by the Olivia variety and the Feteasca neagra 4VI clone in the years 2017-2019
(Source: own determinations and analysis)

CONCLUSIONS

Considering the climatic conditions of the three years (2017-2019) in which the Olivia variety and the Feteasca neagra 4VI clone were monitored, we could conclude that the one that adapted better to the biotic and abiotic factors, in continuous dynamics, was the clone.

The higher productive potential of the Feteasca neagra 4VI clone significantly influenced the yield results obtained, but it cannot be ignored that there is an additional difference of 9-16% in favor of the clone, compared to the Olivia variety.

If these results are going to be confirmed by other research, in the future there is a possibility that clones will be preferred over cultivars, provided that integrated management doesn't involve additional specific operations, as was the case in this study, where all lots have benefited from the same technological conditions.

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EFFECT OF CLIMATE CHANGES ON THE GRAPEVINE PHENOLOGY

Anamaria ILINA (DUMITRU)[1, 2], Dorin SUMEDREA[2], Alina FLOREA[2], Carmen POPESCU[2], Andreea MANOLESCU[2], Sina COSMULESCU[3]

[1]University of Craiova, Faculty of Horticulture, Doctoral School of Plant and Animal Resources Engineering, A.I. Cuza Street, no. 13, 200585, Craiova, Romania

[2]National Research and Development Institute for Biotechnology in Horticulture Stefanesti, Bucuresti - Pitești Street, no. 37, Stefanesti, Arges, Romania

[3]University of Craiova, Faculty of Horticulture, Department of Horticulture and Food Science, A.I. Cuza Street, no. 13, 200585, Craiova, Romania

Corresponding author emails: dsumedrea@yahoo.com, alinaflorea964@gmail.com

Abstract

This research presents a summary of the observed winegrape phenological characteristics and trends. Thirteen wine grape accessions maintained in germplasm collection considered old, autochthonous and neglected genotypes at risk of extinction were analysed for their main phenological evolution. The weather data are recorded from 1979 to 2023, and the phenological data were compared for the last 3-year period. Changes are usually greater at minimum temperatures than maximum temperatures, with an average study-year warming of 1.55°C during the growing season over the multi-year analyzed 1970–2020. Results showed 5 to 14 days earlier budding, 8 to 23 days earlier flowering onset. 'Rară albă' genotypes, the advance for the beginning of flowering was 23 days earlier in 2023 and 21 days earlier in 2022, compared to the beginning recorded in 2021. The advancement of the phenological stages of the vine is similar in other countries with a continental climate and not only, and the risk of the extension of late frosts has increased.

Key words*: grapevine phenophases, climate parameters, Vitis vinifera L., Stefanesti germplasm collection.*

INTRODUCTION

Phenology as a study of phenological events, or the stages of plant development that occur during their active life cycle is in strong relatedness with the climatic conditions from growing area. Any change in the morphological aspect of the plants, or the moment of expression of this change represents, the first biological indicator that highlights the effect of climate change on vine development (Cameron et al., 2021).

Worldwide, the frequency of extreme weather events recorded during the recent decades had a negative influence on the phenology of the grapevine.

The accelerated increase, from year to year, of the average monthly temperatures, as well as the large changes in the amounts and distribution of precipitation, especially during the growing season, had direct effects on the onset and duration of plant development periods, determining new challenges in terms of stress for vine plant adaptation (Bock et al., 2011). The changed climatic conditions strongly affect not only the quantity, but also the quality of harvested grapes, meaning: sugar concentrations, acidity levels in must and wines, and modified varietal aroma compounds.

In addition to climatic conditions, numerous studies have shown that grapevine genotype, vineyard location and pruning techniques are important factors influencing grapevine phenology (Gatti et al., 2016). These separate factors, or as a whole, influence the initiation of budding, flowering, changes in ripening periods, implicitly in the technological activities carried out in the vineyard and finally wine characteristics (Tiffon-Terrade et al., 2023).

For Romania, as in most countries with a millenary tradition in wine production, climate change is a topic of great interest to researchers (Dejeu et al., 2008; Bucur and Dejeu, 2016).

Viticulture in Romania is increasingly faced with climatic phenomena with increased risk to the life cycle of plant culture.
The purpose of the present study is to evaluate the influence of climatic factors (temperature and precipitations) on the main phenological phases of grapevines from *ex situ* Germplasm Collection of NRDIBH Stefanesti, Arges.
The analysis of the stages of annual plant development is important for proper planning of technological activities as well as for maintaining the genetic diversity in the viticultural germplasm collections.

MATERIALS AND METHODS

The study was carried out in the *ex situ* germplasm collection located at the NRDIBH Stefanesti, Arges county. The wine-growing area is characterized by a humid temperate-continental climate, according to the multiannual averages (period 1979-2021), with an average annual temperature (T_{mean}) of 11.06°C and the amount of precipitation of 781.5 mm per year.
From 1979 to 2010, there was a continuous record of weather data with instruments read by a meteorologist four times a day. Since 2010, all measurements were recorded by an IMETOS automated weather station at Spectrum. The weather station is equipped with sensors Watermark Soil Moisture Sensors 6450WD, sensors to read temperature, relative air humidity, wind speed, solar radiation.
The main meteorological indicators in our study, such as minimum and maximum average temperatures, precipitation during the vegetative years, were recorded with the Meteorological Station located in the experimental field.
For this study, were selected 13 white accessions considered valuable and with high risk of extinction, in agreement with Popescu et al. (2017). To record the growth stages of the vine, the BBCH scale known and used by scientists around the world was used (Maier, 2001). The total number of days of vine phenological stages covering the entire cycle,
of growth starting from budding and ending at senescence, was observed: Bud development (BBCH 00-09), Leaf development (BBCH 11-19); Inflorescence emerges (BBCH 53-57); Flowering (BBCH 60-69); Development of fruits (BBCH 71-79); Ripening of berries (BBCH 81-89).
Aiming to highlight the differences among the phenological data recorded during the studied period was applied the Duncan test (test with multiple intervals) for a statistical assurance of 5%. For characteristics phenology and trends were used simple descriptive statistics were calculated for each phenological event.

RESULTS AND DISCUSSIONS

Climate parameters evolution during the grapevine growing seasons

The averages of the annual parameters for the main climate indicators are presented in Table 1. All the data showed that the climate conditions have changed from year to year, becoming overall warmer and drier from year to year. Compared to long-term multiannual averages (1979-2020), the average annual temperature increased by 2.46^0C in 2023.
The maximum temperature in the study years increased by 6.18^0C in 2023, 3.76^0C in 2022 and 1.76^0C in 2021, compared to the maximum that was recorded as an average value in the years 1979-2020. The precipitation deficit recorded was also relevant, with 217.95 less in 2023, 124.45 mm in 2022 and only 10.75 mm in 2021, compared to the multi-year averages.
The large amplitude of meteorological conditions occurring during the vegetation period from 2023 at NRDIBH Stefanesti, has the specificity of an excessively continental climate, this year's vegetation season being a very dry one (with only 563.2 mm compared to the multi-year average of 781.5 mm (Table 1).

Analysis of phenological data from the years of study

The average number of days of the main phenological stages (Sprouting/Bud development BBCH 00-09; Leaf development - BBCH 11-19; Inflorescence emerges -BBCH 53-57; Flowering -BBCH 60-69; Development of fruits -BBCH 71-79; Ripening of berries - BBCH 81-89) for each accession and year were analysed and represented in the Chromatogram from Figure 2. According to our results of the three years of study, the onset of the main vegetation phenophases, as well as the number of days were different even within the same genotype, depending on climatic conditions of each year. Thus, the number of days for the onset of budding varied from 19 days for the 'Muscat tămâios' genotype in 2023, to 26 days for the 'Om rău' and 'Rară albă' genotypes in 2021. A higher average annual temperature in the growing season for 2022 and 2023 influenced both the phenological onset of the genotypes, their flowering and, finally ripening (Figures 1, 2).

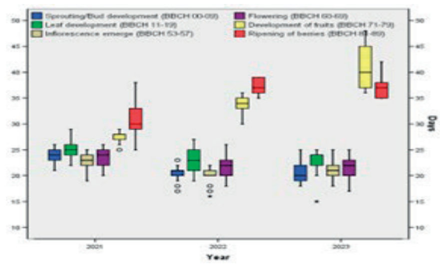

Figure 1. Graphic representation of the phenophases in the cycle of life of the vine depending on the year of study

Table 1. The main climatic parameters registered during the three analysed years (2021-2023) in comparison

Climatic indicator	Multianual average (1979-2020)	2021	2022	2023 (01.01-31.08)
Average annual temperature, ^{0}C	11,06	11,8	12,51	13,52
Average temperature in the growing season, ^{0}C (IV-X)	16,79	17,41	18,27	18,67
Average temperature in summer (°C) (VI-VIII)	21,90	23,08	23,85	30,47
Average annual minimum temperature (°C)	6,87	9,86	6,23	7,84
Absolute minimum temperature (°C)	-17,81	-13,9	-8,4	-10,21
Average January minimum temperature (°C)	-8,84	-10,9	-3,89	-4,69
Average annual maximum temperature (°C)	22,08	23,84	25,84	28,26
Average July maximum temperature (°C)	34,94	37,9	37,21	33,06
Annual total precipitation, mm	781,5	770,4	656,70	563,2
Total precipitation in the growing season, mm (IV-X)	534,13	440,6	559,7	437,8
The total precipitation in summer (VI-IX)	269,27	213,2	234,5	266,4

The trends of the climatic indicators during the three years of the study clearly highlighted the advancement and shortening of the necessary duration for the main phenophases in the development of the studied accessions. The increasing temperatures registered during the growing seasons affected both the onset of the vegetation phenophases and required number of days to reach each development stage, having a significant impact on the growth of the plants. It was noticed that the higher temperatures in the vegetation period of 2023 (Table 1) induced an advance of 5 ('Balaban alb') up to 14 days ('Galbenă măruntă') in terms of the onset of budding in most of the studied genotypes, except for the 'Muscat tămâios' accession, the differences between them being significant (Table 2). They registered an advanced stage of budding 14 ('Pîrciu') and 13 ('Rară albă') days earlier than in 2021, which led to a shortening of the number of days by 6 days and 4 days, respectively. It can be seen that the weather factors of the study years significantly influenced both the flowering period and the duration of flowering (Table 2) in all the analyzed accessions. With high differences regarding the beginning of flowering, the genotypes 'Zghihară rară' and 'Muscat tămâios' also stood out. The climatic conditions of high spring temperature in 2023 resulted in a shorter flowering duration (BBCH 60-69) and the "full flowering" phenophase lasted only a few weeks for the whole range of genotypes. Similar results from the southern part of Romania were also reported by Zaldea et al. (2021), in the northern part of the country.

This may also be due to the phenological progress regarding the first stages (BBCH 00-09).

With 'Balaban alb', 'Rară albă', 'Tigvoasă' and 'Zghihară rară' genotypes, although it was noticed a flowering peak with 8 -26 days earlier in 2022 and 2023 than 2021, the number of days required for full blooming was similar, without significant differences among years. The advance of flowering was 19 days, 15 earlier in the years 2023 and 2022, respectively, compared to 2021 for the "Muscat tămâios" genotype. The full maturity of the grapes evolved depending on the genotype and the climatic conditions of the year.

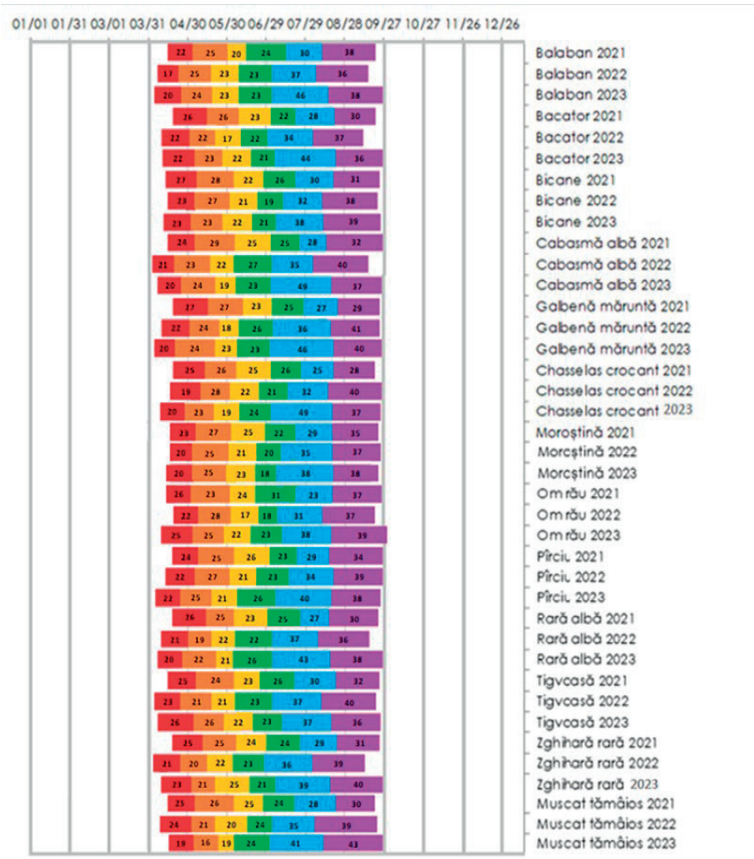

Figure 2. Chromatogram with evolution on the number of days for each genotype and year registered in phenological stages: red/Bud development (BBCH 00-09), orange: Leaf development (BBCH 11-19); yellow: Inflorescence emerges (BBCH 53-57); green: Flowering (BBCH 60-69); blue: Development of fruits (BBCH 71-79); purple: Ripening of berries (BBCH 81-89)

The ripening phase lasted an average of 39 days, for all the analyzed accessions. The shortest number of days regarding ripening was registered for 'Rară albă' with 30 days in 2021, and the longest for 'Muscat tămâios' with 43 days elapsed, in 2023. For all the analyzed accessions, the number of days for ripening phase was significantly different over the 3 years, with the fewest days registered in 2021.
Also, significant changes in the harvest dates were highlighted for some studied genotypes where harvesting starts earlier (Table 3).
With a phenological advance of 8 days in 2023 and 9 days earlier in 2022 than in 2021, the 'Bicane' genotype had an extension of days regarding ripening, the differences being significant. The same very significant differences were also revealed in the case of the genotypes 'Pîrciu', 'Rără albă', 'Tigvoasă', 'Zghihară rară', 'Muscat tămâios'. For all these genotypes, the length of the number of days was extended by 9 days for 'Zghihară rară' genotype in 2023, respectively 8 in 2022, up to 13 days in the case of the 'Muscat tămâios' genotype, in 2023, compared to the data recorded in 2021 (Table 3).
The extension of the ripening period of the analyzed grapes, especially in the years 2022 and 2023, can be due on the one hand to the lower temperatures during the nights in September, and, on the other hand, to the higher rainfall during this period, compared to the year 2021.

Table 2. Evolution of principal phenophase stages (BBCH 00-09 and BBCH 60-69) from the three years of analysis

Genotypes	**Indicators**	**Sprouting/Bud development (BBCH 00-09)**			**Flowering (BBCH 60-69)**		
		2021	**2022**	**2023**	**2021**	**2022**	**2023**
'Balaban alb'	Variation limit	April 15	April 07	April 05	June 21	June 11	June 11
		May 06	April 23	April 24	July 14	July 03	July 03
	No. of days/ phenophase	22±0.22[b]	17±-0.41[a]	20±0.36[b]	24±0.23[a]	23±0.62[a]	23±0.35[a]
'Bacator'	Variation limit	April 19	April 10	April 13	July 03	June 10	June 19
		May 14	May 01	May 04	July 24	July 01	July 08
	No. of days/ phenophase	26±-.41[b]	22±0.22[a]	22±0.34[a]	22±0.33[a]	22±0.26[a]	21±0.43[a]
'Bicane'	Variation limit	April 13	April 15	April 12	June 29	June 25	June 19
		May 09	May 07	May 04	July 24	July 13	July 08
	No. of days/ phenophase	27±0.51[b]	23±0.23[a]	23 ±0.44[a]	26±0.48[b]	19±0.12[a]	21±0.32[a]
'Cabasmă albă'	Variation limit	April 15	April 03	April 07	July 03	June 08	June 09
		May 08	April 23	April 26	July 27	July 04	July 02
	No. of days/ phenophase	24±0.12[ab]	21±0.42[a]	20±0.35[a]	25±0.23[a]	27±0.19[ab]	23±0.41[a]
'Galbenă măruntă'	Variation limit	April 19	April 10	April 05	July 05	June 13	June 11
		May 15	May 01	April 24	July 29	July 08	July 03
	No. of days/ phenophase	27±0.48[b]	22±0.15[a]	20±0.35[a]	25±0.41[a]	26±0.29[b]	23±0.39[a]
'Chasselas crocant'	Variation limit	April 19	April 17	April 07	July 03	June 25	June 09
		May 13	May 05	April 27	July 28	July 15	July 02
	No. of days/ phenophase	25±0.16[b]	19±0.56[a]	20±0.23[a]	26±0.17[ab]	21±0.22[a]	24±0.60[a]
'Moroștină'	Variation limit	April 17	April 17	April 14	July 01	June 23	June 23
		May 09	May 06	May 04	July 22	July 12	July 10
	No. of days/ phenophase	23±0.33a	20±0.33a	20±0.47a	22±0.48[b]	20±0.39[a]	18±0.38[a]
'Om rău'	Variation limit	April 15	April 20	April 10	June 27	June 26	June 21
		May 10	May 11	May 04	July 27	July 13	July 13
	No. of days/ phenophase	26±0.22[b]	22±0.46[a]	25±0.12[b]	31±0.44[b]	18±0.32[a]	23±0.41[a]
'Pîrciu'	Variation limit	April 19	April 14	April 06	July 03	June 23	June 13
		May 12	May 05	April 27	July 25	July 15	July 08
	No. of days/ phenophase	24±0.51[a]	22±0.32[a]	22±0.26[a]	23±0.36[a]	23±0.41[a]	26±0.25[a]
'Rară albă'	Variation limit	April 19	April 10	April 07	July 02	June 11	June 09
		May 14	April 30	April 26	July 26	July 04	July 04
	No. of days/ phenophase	26±0.44[b]	21±0.42[a]	20±0.39[a]	25±0.21[a]	22±0.46[a]	26±0.12[ab]
'Tigvoasă'	Variation limit	April 1	April 05	April 08	June 27	June 09	June 21
		May 09	April 27	May 03	July 22	July 03	July 13
	No. of days/ phenophase	25±0.32[b]	23±0.66[a]	26±0.18[b]	26±0.23[a]	23±0.46[a]	23±0.42[a]
'Zghihară rară'	Variation limit	April 19	April 04	April 10	July 02	June 06	June 18
		May 13	April 24	May 02	July 25	June 29	July 08
	No. of days/ phenophase	25±0.44[b]	21±0.38[a]	23±0.25[a]	24±0.22[a]	23±0.56[a]	21±0.38[a]
'Muscat tămâios'	Variation limit	April 15	April 10	April 16	June 30	June 15	June 09
		May 09	May 03	May 04	July 23	July 08	July 02
	No. of days/ phenophase	25±b	24±b	19±a	24±0.56[a]	24±0.41[a]	24±0.50[a]

Table 3. Evolution of principal phenophase stages (BBCH 71-79 and BBCH 81-89) from the three years of analysis

Genotypes	Indicators	**Development of fruits (BBCH 71-79)**			**Ripening of berries (BBCH 81-89)**		
		2021	**2022**	**2023**	**2021**	**2022**	**2023**
'Balaban alb'	Variation limit	July 15	July 04	July 04	August 14	August 10	August 19
		August 13	August 09	August 18	September20	September14	September 25
	No. of days/ phenophase	30±0.32[a]	37±0.51[ab]	46±0.34[b]	38±0.19[a]	36±0.66[a]	38±0.42[a]
'Bacator'	Variation limit	July 25	July 02	July 09	August 22	August 05	August 22
		August 21	August 04	August 21	September20	September10	September 26
	No. of days/ phenophase	28±0.55[a]	34±0.31[b]	44±0.28[b]	30±0.44[a]	37±0.40[a]	36±0.31[a]
'Bicane'	Variation limit	July 25	July 14	July 09	August 24	August 15	August 16
		August 23	August 14	August 15	September23	September21	September 23
	No. of days/ phenophase	30±0.25[a]	32±0.17[ab]	38±0.49[b]	31±0.56[a]	38±0.49[b]	39±0.28[b]

'Cabasmă albă'	Variation limit	July 28	July 05	July 03	August 25	August 09	August 21
		August 24	August 08	August 20	September25	September17	September 26
	No. of days/ phenophase	28±0.51^{a}	35±0.42^{b}	49±0.23^{b}	32±0.48^{a}	40±0.35^{b}	37±0.23^{ab}
'Galbenă măruntă'	Variation limit	July 30	July 09	July 04	August 26	August 14	August 19
		August 25	August 13	August 18	September23	September23	September 27
	No. of days/ phenophase	27±0.58^{a}	36±0.39^{b}	46±0.28b	29±0.66^{a}	41±0.42^{b}	40±0.31^{b}
'Chasselas crocant'	Variation limit	July 29	July 16	July 03	August 23	August 17	August 21
		August 22	August 16	August 20	September19	September25	September 26
	No. of days/ phenophase	25±0.51^{a}	32±0.54^{b}	49±0.41^{c}	28±0.46^{a}	40±0.39^{b}	37±0.25^{b}
'Moroștină'	Variation limit	July 23	July 13	July 11	August 21	August 17	August 18
		August 20	August 16	August 17	September24	September22	September 24
	No. of days/ phenophase	29±0.64^{a}	35±0.40^{b}	38±0.31^{b}	35±0.33^{a}	37±0.58^{a}	38±0.55^{a}
'Om rău'	Variation limit	July 28	July 14	July 14	August 20	August 14	August 21
		August 19	August 13	August 20	September25	September19	September 28
	No. of days/ phenophase	23±0.45^{a}	31±0.33^{b}	38±0.24^{c}	37±0.44^{a}	37±0.19^{a}	39±0.27^{a}
'Pîrciu'	Variation limit	July 26	July 16	July 09	August 24	August 19	August 18
		August 23	August 18	August 17	September26	September26	September 24
	No. of days/ phenophase	29±0.33^{a}	34±0.42^{ab}	40±0.18^{b}	34±0.48^{a}	39±0.35^{b}	38±0.22^{b}
'Rară albă'	Variation limit	July 27	July 05	July 05	August 23	August 11	August 17
		August 22	August 10	August 16	September21	September15	September 23
	No. of days/ phenophase	27±0.27^{a}	37±0.38^{b}	43±0.55c	30±0.48^{a}	36±0.45^{b}	38±0.30^{b}
'Tigvoasă'	Variation limit	July 23	July 04	July 14	August 22	August 12	August 20
		August 21	August 11	August 19	September22	September20	September 24
	No. of days/ phenophase	30±0.55^{a}	37±0.38^{ab}	37±0.24^{ab}	32±0.46^{a}	40±0.39^{b}	36±0.27^{ab}
'Zghihară rară'	Variation limit	July 26	July 30	July 09	August 24	August 04	August 17
		August 23	August 03	August 16	September23	September11	September 25
	No. of days/ phenophase	29±0.62^{a}	36±0.34^{b}	39±0.24^{c}	31±0.51^{a}	39±0.41^{b}	40±0.22^{b}
'Muscat tămâios'	Variation limit	July 24	July 09	July 03	August 21	August 13	August 13
		August 20	August 12	August 12	September19	September20	September 24
	No. of days/ phenophase	28±0.51^{a}	35±0.41^{b}	41±0.23^{b}	30±0.44^{a}	39±0.38^{b}	43±0.23^{b}

Relationship between climatic condition and phenological trends

The descriptive statistical indicators and the trends of phenological, of the corresponding stages and intervals of the 13 genotypes resulting from the data recorded in the 3 years, are presented in Table 4.

The average date of the thirteen analyzed genotypes for the onset of budding was April 12 with an interval of 22 days to complete. The earliest genotypes were 'Cabasmă albă', 'Balaban alb' and 'Galbenă măruntă' which budded on April 8 and 12 respectively, with a length of 21 and 22 days respectively. The latest genotypes were 'Chasselas crocant' and 'Moroștină', where the beginning of budding was on average, April 16.

According to the descriptive statistics, the beginning of budding showed the significant trends for some of the studied genotypes, with the exception of the genotypes 'Balaban alb', 'Bicane', 'Moroștină', 'Om rău', 'Tigvoasă', 'Zghihară rară', and 'Muscat tămâios'.

The average date for the onset of flowering, on average across all genotypes, has a trend of 0.4 days/year.

The strongest linear trend of the phenophase at the beginning of flowering was observed in the genotypes 'Cabasmă albă', 'Rară albă' and 'Zghihară rară'. The phenological advance was for these genotypes over 0.7 days/year, and the lowest advance, with approximately 0.3 days/year, was recorded for the genotypes 'Moroștină' and 'Muscat tămâios'.

Table 4. Statistical and trends indicators descriptive for the phenophases of the beginning of budding (BBCH 00-09) and the flowering (BBCH 60) in the period 2021-2023.

Phenological stage	Beginning of budburst/Bud development (BBCH 00-09)						Beginning of Flowering (BBCH 60)					
Statistics indicators (dates and days)	Mean	Range	S.D	Tend	R^2	P	Mean	Range	S.D.	Tend	R^2	P
'Balaban alb'	9 April	21	6.5	0.3	0.09	NS	14 June	23	6.8	0.15	0.08	NS
'Bacator'	14 April	23	7.14	**0.38**	0.42	<0.05	15 June	23	7.2	**0.67**	0.18	<0·001
'Bicane'	13 April	24	8.8	0.24	0.04	NS	24 June	22	9.3	0. 21	0.09	NS
'Cabasmă albă'	8 April	22	5.9	**0.31**	0.38	<0.05	7 June	25	8.4	**0.78**	0.13	<0.01
'Galbenă măruntă'	12 April	22	1.2	**0.21**	0.12	<0·001	10 June	25	4,8	**0.54**	0.12	<0.05
'Chasselas crocant'	16 April	21	5.1	**0.3**	0.41	<0.05	19 June	24	9,8	**0.62**	018	<0·05
'Moroștină'	16 April	21	3.1	0.15	0.02	NS	21 June	20	7.2	**0.34**	0.19	<0·001
'Om rău'	15 April	23	7.3	0.17	0.08	NS	24 June	24	8.1	0.12	0.22	NS
'Pîrciu'	13 April	23	6.4	**0.23**	0.30	<0·001	16 June	24	6.6	**0.48**	0.19	<0.05
'Rară albă'	12 April	22	10.3	**0.68**	0.33	<0·001	19 June	23	4.8	**0.75**	0.22	<0.01
'Tigvoas'	10 April	22	5.3	0.3	0.02	NS	18 June	24	7.8	0.31	0.06	NS
'Zghihară rară'	13 April	23	9.1	0.17	0.07	NS	14 June	23	9.9	**0.71**	0.21	<0·001
'Muscat tămâios'	14 April	23	5.6	0.25	0.09	NS	19 June	24	8.8	**0.38**	0.12	<0·001

The high temperatures, especially in the first part of the year, obviously affect the period of budding and flowering, and less so the ripening phase. Neethling et al. (2012) report shorter ripening rates (0.3 days/year), with long rates of flowering advance to vine. On the average of the years of study, the intensity of the correlations between the phenophases of the vine, in the genotypes analyzed, presented in Table 5, showed that, due to the phenological advance regarding budding, there was also an advance for the Flowering (BBCH 60-69). Significant positive correlations were found for these (r= 0.377***).

Table 5. Correlation matrix between the phenophases of the vine

Pearson Correlation	Flowering (BBCH 60-69)	Development of fruits (BBCH 71-79)	Ripening of berries (BBCH 81-89)
Sprouting/Bud development (BBCH 00-09)	0.377 (***)	-0.647 (**)	-0.664 (**)
Sig. (2-tailed)	0.018	0.000	0.000
Sig. (2-tailed)	0.280	0.029	0.009
Flowering (BBCH 60-69)	1	-0.184	-0.319 (*)
Sig. (2-tailed)		0.261	0.048

A negative and significant correlation is observed between the flowering phase and fruit ripening (r= -0.319*). Even if, in most genotypes, the flowering phase lasted less, the number of ripening days was extended.

CONCLUSIONS

The climatic parameters recorded in the Stefanesti germplasm collection during the three consecutive years (2021-2023) affected all phenological growth development of the plants starting from budding and ending to the technological harvested moment. Each genotype responded differently to changes in the main climatic factors, being proof of their different plasticity of response and adaptation to climatic changes.
The following trends were highlighted:
- a trigger of budding earlier with 3 days for 'Bicane' and 'Moroștină' accessions up to 14, respectively 15 days in the case of 'Galbenă măruntă' and 'Zghihară rară' accessions;
- on the average of the years studied, the date of the start of flowering for all genotypes had an advance trend of 0.4 days/year. The significant trend was observed with 'Cabasmă albă', 'Rară albă' and 'Zghihară rară' genotypes, with an advance of 0.7 days/year, and the lowest tend for 'Moroștină' and 'Muscat tămâios' accessions with an advance more than 0.3 days/year;
- the 'Rară albă' genotype had the shortest number of days to ripen with 30 days in 2021,

and the longest for the 'Muscat tămâios' genotype with 43 days passed, in 2023.

For the future, knowing the stages of development and the different adaptation responses of the grapevine in the context of climate change will be useful to reconsider the old and autochthonous genotypes. Moreover, this plant material could be essential to be used in specific breeding programs, or as proper grapevine genotypes in specific growing areas.

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ANTHOCYANINS LEVELS MODIFICATION ON WINES WITHOUT SULFUR DIOXIDE. NEW PERSPECTIVE.

Sorin MACOVICIUC[1], Bogdan-Ionel CIOROIU[2], Marius NICULAUA[2], Constatin-Bogdan NECHITA[2], Valeriu V. COTEA[1, 2]

[1]Iasi University of Life Sciences “Ion Ionescu de la Brad”, 3 M. Sadoveanu Alley, Iasi, Romania
[2]Romanian Academy - Iasi Branch, Research Centre for Oenology,
9H M. Sadoveanu Alley, Iasi, Romania

Corresponding author email: bogdan.cioroiu@acadiasi.ro

Abstract

Anthocyanins are considered important compounds in wine production. Their role is important in wine color in case of red and rose wines. Anthocyanins are involved all stages of wine production from grapes, fermentation, treatment and aging. Wine evolution produce modification of pH media, color or other physico-chemical parameters. Principal characteristics as aromas are determined by several reactions as acido-basic and redox which are related to modifications of wine composition. An UFLC method was involved in evaluation of several compounds as Cyanidol-3-glucoside (Cy3gl), delphidin (Dp), peonidin 3-glucoside (Po3gl), malvidin 3-glucoside (Mv3gl), petunidin (Pt) and malvidin (Mv) which were separated and analyzed in case of several wine samples from Cabernet Sauvignon and Cabernet Sauvignon rose. Comparative profiles showed significant differences in levels of these compounds in case of the wines treated with sulphur dioxide and without sulphur dioxide. Conclusion is that use of natural compounds as Pichia kluyveri yeasts not only prevented oxidation with implications in anthocyanins variation but showed potency against bacterial contamination.

Key words: *wines, sulphur dioxide, anthocyanins, UFLC.*

INTRODUCTION

Through Pichia variants, *P. kluyveri* is the most studied and is the only available on commercial level. *P. kluyveri* has the potential to produce aromatic compounds as thiols, terpenes and several esters (Vicente et al., 2021). Among the commercial products, Frootzen® (Hansen®, Hoersholm, Danemarck) is recognized with the capacity of improve varietal and thiols aromas (Vejarano and Gil-Calderón, 2021). There is a concern regarding the wine alteration in presence of non-*Saccharomices* species, thus special consideration should be taken in order to be efficient and viable for inhibition of fermentation–yeasts (Vejarano, 2020). For this purpose, at wide scale sulphur dioxide is used for antimicrobial effect, but the extended use may produce allergic reaction and other health disorders.

Several reactions are encountered during wine aging, one of most importance is oxidation which is produced at the levels of compounds susceptible to be sensible and in this category are included also anthocyanins (Deshaies et al., 2020). The stability of monomeric anthocyanin in red wines is influenced by grape variety, fermentation techniques or wine management (Baiano et al., 2016). Over influences are related to pH value, temperature, oxidation state, exposure to light, presence of other substances such as ascorbic acid, sugars, sulphites and metal ions as cofactors (He et al., 2012). Normally, anthocyanins appear to be more stable in acidic media at lower pH values than in alkaline solutions with higher pH values.

Involved in wine colour, anthocyanins are influenced by the presence of other compounds as flavanols or tannins. As function of their structure, anthocyanins are classified in more groups: anthocyanins non-acylated, acetylated anthocyanins, proanthocyanins, anthocyanins – flavanol, acetyl mediated condensation flavanol anthocyanins (Pervaiz et al., 2017).

The objective of this study is to evaluate the chemical substances and sensory impact of ome commercial yeast product of *non-Saccharomyces* yeasts used for bio-protection.

MATERIALS AND METHODS

Wine preparation procedures

The study is based on Cabernet Sauvignon grape sort from Panciu wine region with the following coordinates 45°52'52.3"N and 27°03'23.4"E. Two classical wine types were included in the study, each with samples without sulphur dioxide and control samples that had added SO_2 in doses of up 50 mg/L.

Namely, Cabernet Sauvignon rose (CSr) and Cabernet Sauvignon red (CS) were vinified in two wine making procedures. Representative samples were taken in 2018, 2019, 2020 and 2021 and stored in wine cellar for aging and codified in the following manner: CS - Cabernet Sauvignon, CSr - Cabernet Sauvignon rose, 0-(-SO_2 samples), 1-(+SO_2 samples), year - (18, 19, 20, 21) → (2018, 2019, 2020, 2021).

In every of each year, vinification of CS0 and CSr0 started with grape treatment with *P. kluyveri* in doses of 1.3 kg/T while for CS1 and CSr1 wines grape treatment consisted in use of potassium metabisulphite (6 g/100 kg), ascorbic acid (3 g/100 kg) and gallic acid (1 g/100 kg).

Maceration was produced for maximum 3 hours for CSr0 and CSr1, followed by must separation and destemming using pectolytic enzymes (3 g/hL). Alcoholic fermentation used promotors in two stages and doses of 25 g/L followed by *S. cerevisiae* (20 g/hL). CSr0 had malolactic fermentation 2 days after alcoholic debut using standard *O. Oeni. yeast* with nutrient (20 g/hL) to avoid any refermentation of wine.

CS0 and CS1 wines had alcoholic (*S. cerevisiae* - 20 g/hL) and malolactic fermentation (*O. Oeni.* in presence of fermentation nutrient - 20 g/hL) concomitant with maceration (pectolytic enzymes - 3.0 g/hL), inactivated yeasts and autolysates - 25 g/hL)). All the wines had discontinuous press and decanting for separation from deposits. Sulfur dioxide variants had SO_2 correction (up to 40 mg/L free SO_2) while samples without SO_2 had an antioxidant treatment with oenological tannins 5 g/hL. Both type of samples had acidity correction with lactic and tartaric acid up to 6.1-6.3 g tartaric acid/L. CSr0 and CSr1 samples had polyphenols reduction by using PVPP (15 g/hL) and clarification with bentonite (100 g/hL), and fish clay (25 ml/hL). Tartaric stabilization was made with potassium polyaspartate (200 ml/hL) for CS while CSr wines were treated with carboxymethylcellulose (100 g/hL) and arabic gum (100 g/hL). Final aromatic complexity was assured using mannoproteins (3 g/hL) (CS) and condensed tannins (0.5 g/hL) (CS and CSr), while CS1 and CSr1 had final SO_2 correction before bottling.

Reagents and reference materials

For analysis of anthocyanins, formic acid, acetonitrile (LC grade) were used and purchased from Merck KGaA - Germany. Cyanidin 3-glucoside chloride (koumarin hydrochloride) (Cy3gl - CAS 7084-24-4), delphidin chloride (Dp - CAS 208-437-0), peonidin 3-glucoside chloride (Po3gl - CAS 6906-39-4), malvidin 3-glucoside chloride (Mv3gl - CAS 7228-78-6), petunidin chloride (Pt - CAS 1429-30-7) and malvidin chloride (Mv - CAS 643-84-5) were used as standards and procured from Extrasyntesis France. For UFLC analysis a type I water produced by a Thermo Scientific GenPure UV-TOC system.

Instrumental methods

A high-performance chromatography method was applied using an Agilent 1220 series. As chromatographic column a C18 Phenomenex with 150 mm length, 4.6 mm internal diameter and 2.6 µm was used. Stationary phase consisted in superficial porous particles with 100Å size (SPP - Kinetex XB - C18, 00F-4496-E0) pre-column (AJ0-8768). Chromatographic elution was gradient and used a mixture of Water/Formic acid/Acetonitrile. in the following parts-per-volume 87:10:3 (v/v/v) (Solvent A) and 40:10:50 (v/v/v) (Solvent B). Flowrate was 1.85 mL/min, temperature 50°C and injected volume was 10 µL. Detection was at 525 nm, 1 nm slit and 80 Hz (0.13 s) data sampling rate.

Statistical analysis

All the statistical analysis were performed using StatSoft Tibco Statistica. Univariate analysis of variance was applied to make a comparison between the levels of biogenic amines in the case of different wine varieties, but also between the wines which are treated with and without SO_2. When different values of the concentrations were registered, LSD (Least Significant Difference) test was applied to determine which mean values have significant differences.

RESULTS AND DISCUSSIONS

Instrumental evaluation

Chromatographic method followed the conditions from reference methodologies (OIV-MA-AS315-11), but with slight modifications in terms of elution and flowrate. Reference method used a flowrate of 0.8 mL/min on a SPP chromatographic column with a particle size of 2.6 µm (0.35 µm porous shell and 1.9 µm solid core) and achieved a 41-minute gradient program. Significant improvement was produced using our conditions in terms of lower chromatography time and optimum resolution for every compound. In terms of gradient, composition started with solvent A:B 94:6 (v/v), changed to 70:30 (v/v) in 4.5 minutes and 40:60 (v/v) in 7 minutes. Re-equilibration was achieved in 2 minutes and total chromatography time was 9 minutes. Retention times (min) were Cy3gl (2.26), Dp (2.72), Po3gl (3.39), Mv3gl (3.91), Pt (4.26), Pg (4.64), and Mv (5.77). Correlation coefficient for every slope was of no less than 0.99. Limit of quantification ranged between 3.2 µM for Dp and 43.5µM for Mv.

Sample evaluation during the study

Representative samples were identified as previously mentioned. After sampling all the specimens were stored in cellar, in conditions which assured minimization of any supplementary oxidation to the samples. Also, representative analysis was taken to verify the levels of fixed acidity and content of sulphur dioxide. For the CS1 and CSr1 wines, the levels of free sulphur dioxide ranged between 40-58 mg/L. samples without added sulphur dioxide as CS0 and CSr0 the quantities were not higher than 6 mg/L and originated from fermentation stage (Pezley 2015). All the samples were stored for specified time interval (2018-2021) and the final analysis for the anthocyanins was performed on 2021.

pH was important for stability of wines and maintained between 3.1 and 3.3 during winemaking. pH, acidity, alcoholic content, volatile acidity, sugar content were monitored to verify the primary conditions that could lead to anthocyanins alterations. Regarding pH, CS showed values between 3.0 and 3.74 for CS0 and 2.91 to 3.72 for CS1 samples. Same evaluation was done for CSr: variations of 3.12-3.51 were registered for CSr0 samples and 3.38-3.82 for CSr1 samples. pH modification is determined by organic acids variation. Increase of pH which was correlated with slight decrease of fixed acidity and is determined by precipitation of tartaric acid which is considered unstable (Vicente et al., 2022). Variation of pH did not show any statistical differences between these samples. Anthocyanins are be more stable in acidic media at lower pH values than in alkaline solutions. Redox reactions are favored by modification of oxidation state directly related with change of ionization status (Wahyuningsih et al., 2016). In these conditions, no wine samples showed conditions for important oxidative reactions. Although stabilization was considered efficient, some changes in concentrations of anthocyanins were produced for CS and CSr samples. Variations are related with polymerization and slight oxidative condensation (Cotea et al., 2009). Data is presented in the Tables 1a and 1b.

Table 1a. Levels of anthocyanins for Cabernet Sauvignon rose (CSr) (#1 - CSr018, #2 - CSr019, #3 - CSr020, #4 - CSr021, #5 - CSr118, #6 - CSr119, #7 - CSr120, #8 - CSr121; Av (CSr0 and CSr1) - average value, St - standard deviation)

Anthocyanins (µmols/L) Cabernet Sauvignon rose (CSr)							
#	*Cy3gl*	*Dp*	*Po3gl*	*Mv3gl*	*Pt*	*Mv*	*Total*
1	12.5	6.2	1228	1503	11.7	51.3	2813
2	12.2	4.2	1252	1524	12.9	43.4	2849
3	13.1	3.3	1251	1671	14.2	44.1	2997
4	15.2	6.3	1229	1454	7.4	85.0	2797
Av	***13.3***	***5.0***	***1240***	***1538***	***11.6***	***56.0***	***2864***
St	***1.4***	***1.5***	***13.3***	***93.4***	***2.9***	***19.7***	***91.2***
5	8.5	3.3	1250	1399	6.8	58.2	2726
6	9.1	4.4	1253	1423	8.9	62.1	2761
7	9.2	6.2	1258	1443	12.0	58.8	2787
8	9.3	4.5	1261	1429	9.8	62.0	2776
Av	***9.0***	***4.6***	***1256***	***1424***	***9.4***	***60.3***	***2762***
St	***0.4***	***1.2***	***4.9***	***18.4***	***2.2***	***2.1***	***26.7***

Table 1b. Levels of anthocyanins for Cabernet Sauvignon (CS) (#1' - CS018, #2' - CS019, #3' - CS020, #4' - CS021, #5' - CS118, #6' - CS119, #7' - CS120, #8' - CS121; Av (CS0 and CS1) - average value, St - standard deviation)

Anthocyanins (mmols/L) Cabernet Sauvignon (CS)							
#	*Cy3gl*	*Dp*	*Po3gl*	*Mv3gl*	*Pt*	*Mv*	*Total*
1'	0.33	0.59	2.1	9.2	0.35	0.73	13.3
2'	0.33	0.59	2.2	13.5	0.41	0.83	17.9
3'	0.34	1.15	2.1	23.5	0.44	2.39	29.9
4'	0.35	1.33	2.2	22.9	0.53	2.42	29.7
Av	***0.34***	***0.91***	***2.1***	***17.3***	***0.43***	***1.59***	***22.7***
Sd	***0.01***	***0.38***	***0.06***	***7.05***	***0.07***	***0.94***	***8.40***
5'	0.25	0.41	2.0	8.6	0.39	0.54	12.2
6'	0.26	0.45	1.9	10.7	0.41	0.54	14.3
7'	0.49	1.31	2.5	25.0	0.49	1.63	31.4
8'	0.50	1.36	2.5	25.5	0.52	1.67	32.0
Av	***0.37***	***0.88***	***2.2***	***17.5***	***0.45***	***1.10***	***22.5***
st	***0.14***	***0.52***	***0.29***	***9.04***	***0.06***	***0.64***	***10.68***

According to previous data, in terms of Cabernet Sauvignon rose, no statistical differences were registered for any compound included in the study at the comparison between treatments. Only for Cy3gl the mean value was 13.3 ± 1.4 μmol/L for CSr0, while CSr1 samples showed a significant lower value of 9.0 ± 0.4 μmol/L.
The other anthocyanins ranged between 1424 ± 18.4 μmol/L (Mv3gl; CSr1) and 4.6 ± 1.2 μmol/L (Dp, CSr1). As in other studies, Po3gl and Mv3gl are the major constituents as pigmentation compounds in red wines.
CS varieties had similar characterization but with higher levels and relative content (Fernandes et al., 2017). Mv3gl and Po3gl were the major constituents in red wine pigmentation, followed by Pt, but also Mv and glycolysis form as Mv3gl (Table 1b).
Since the spectra of color compounds is the same and the statistical differences between (CS0, CSr0) and control samples (CS1, CSr1) are not statistically significant, the influence and impact of antioxidants are verified as function of variation during wine aging (Obreque-Slier et al., 2023).
For CS, modification of color was more intense than the CSr, aspect that was confirmed by the slightest variation of the anthocyanins, as showed in Figure 2b.
For CSr, several compounds had a better stability, showed by the Figure 2a where the trendline have correlation coefficients of maximum 0.832. Cy3gl and Po3gl showed a clear evolution during aging period of wine from 2018 and 2021 with r (coefficient of correlation) values of 0.913 and 0.995. Statistical differences between samples at treatment comparison did not show any relevance for every compound in the study.
Continuous evolution of compounds showed statistical differences at comparison using year of production (from 2018 → 2021) as criteria. Only Cy3gl, Po3gl had constant values for which Pearson coefficients have values higher than 0.05.

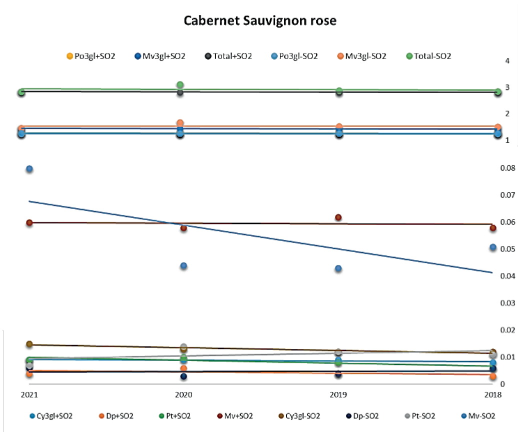

Figure 2. a) Evolution of anthocyanin content for CSr samples

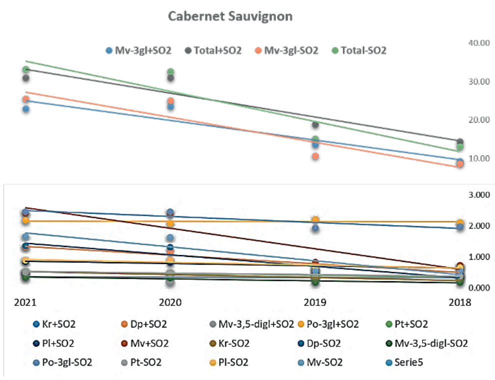

Figure 2b) Evolution of anthocyanin content for CS samples

In direct correlation with low values of correlation coefficients, there are also the mean values of anthocyanins concentrations at treatment comparison, which were not significant different, as mentioned previously, exception was Cy3gl with average value was

13.3 ± 1.4 μmol/L CSr0 against 9.0 ± 0.4 μmol/L (CSr1) which is significant lower. For CS samples the coefficient of determinations have positive values which show a decrease concentration of some anthocyanins as Cy3gl, Dp, Mv3gl, Pt, Mv or total content because the highest values were registered for 2021 and the lowest values were found for 2018 sample, indifferent of treatment (Table 2).

Table 2. Correlation coefficients between levels of anthocyanins and year of production (aging period) (marked values corresponds to $p<0.05$)

Treatment	**-SO$_2$**		**+SO$_2$**	
Sample	**CS0**	**CSr0**	**CS1**	**CSr1**
Cy3gl	0.905	0.913	0.924	0.775
Dp	0.914	**-0.086**	0.940	**0.513**
Po3gl	0.872	**0.019**	0.158	0.995
Mv3gl	0.925	**0.000**	0.933	0.774
Pt	0.979	**-0.439**	0.983	0.832
Mv	0.902	0.654	0.912	0.135
Total	0.923	0.154	0.943	0.914

CONCLUSIONS

Present study created the premises for evaluation of wine stability and evolution during aging period in case of red and rose Cabernet Sauvignon wines. Most important compounds showed clear behavior during wine storage and produced two different evolutions. Some of the compounds as Po3gl, Mv3gl, Cy3gl and Dp had a higher stability in terms of concentration modification, especially for rose wine as confirmed by other studies used as reference. In exchange, glycosides forms presented an instability in terms of decrease, fact that was confirmed by structural modification determined by their functions and biosynthetic pathways.

For red wines, the levels were superior to rose samples and had a different behavior in terms of decrease of anthocyanins during monitorization time. Important is that the samples without sulphur dioxide presented a slight superior stability since Po3gl had a stable concentration. From the perspective of treatment comparison, the two types of wine presented similar behavior, so the vinification technology achieved the goal of producing reliable wines from the perspective of minimization the oxidative reactions that could alter wine quality. Further studies are required in case of new technology to produce wines without added sulphur dioxide. Considering the modification of anthocyanins during wine aging, evaluations for compounds as condensation and polymerization forms will be realized.

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HARVEST AND QUALITY OF GRAPES OF DIFFERENT CLONES OF THE CABERNET SAUVIGNON VARIETY IN THE CODRU WINE-GROWING REGION OF THE REPUBLIC OF MOLDOVA

Gheorghe NICOLAESCU, Olga MOGÎLDEA, Cornelia VOINESCO, Mariana GODOROJA, Gheorghe MATCU, Dumitru VARTIC, Andrei KIMAKOVSKI

Technical University of Moldova, 48, Mircesti Street, Chisinau, Republic of Moldova

Corresponding author emails: gheorghe.nicolaescu@fh.utm.md, gh.nicolaescu@gmail.com

Abstract

The improvement of grape varieties is inevitable in increasing the yield of grapes and the quality of wine. A wide range of clones within each grape variety are currently highlighted. The purpose of our research is to study the behavior of different clones of the Cabernet Sauvignon variety in the Mereni wine-growing area, the Codru wine-growing region of the Republic of Moldova. The Mereni wine-growing area includes the localities of the Anenii Noi district (approx. 30 km SE from the Chisinau city), it is characterized by uneven, warm winters and dry summers with low precipitation. The studied clones demonstrate the adaptability to the conditions of the Codru wine region and the obtaining of high quality wines.

Key words: *clones, Republic of Moldova, viticulture, wine varieties.*

INTRODUCTION

The grape variety have an important role on the quality of the wine, as it determines the organoleptic characteristics of the wines, such as aroma, taste, colour and body. Each grape variety has its own characteristics and can therefore produce a wine with a unique taste, aroma and texture. Among the factors that influence the quality of the wine are: pedoclimatic conditions, growing technology, but the grape variety has a major impact on the characteristics of the wine. In addition, how the grapes are handled and the winemaking process can also influence the final quality of the wine. But without a proper choice of grape variety, it is very difficult to get a top-quality wine (Kimakovski et al., 2023; Кимаковски et al., 2023; Voinesco et al., 2023).

The development of the grapes and winemaking sector is influenced by a series of subjective and objective factors of different origins: technological, ecological, political, informational, financial, etc. (Midari et al., 2022).

Moldova is a country with a tradition in wine production and is known throughout the world for the quality of its wines. The grape and winemaking sector is strategic for the Republic of Moldova economy. The wine-growing regions of Moldova are located in the central and southern part of the country (Nicolaescu et al., 2022; Nicolaescu et al., 2023)

The following wine regions are approved by law:

Codru - is considered the most important wine region in Moldova. The wines produced in this region are known for their full and balanced aroma and taste.

Valul lui Traian - located in the southern part of the republic. It is known for its wines, with delicate aromas of flowers and fruits.

Stefan Voda - located in the southeastern part of Moldova, this region is known for its strong and elegant red wines, produced from varieties such as Cabernet Sauvignon, Merlot and Pinot Noir, etc. (Mogîldea et al., 2023).

Cabernet Sauvignon is a grape variety native from the Bordeaux region of France, but which is successfully grown around the world. Cabernet Sauvignon is a red grape variety and is considered one of the noblest grape varieties due to its organoleptic qualities and the ability to produce wines with the potential for long period of maturation. The wines from Cabernet Sauvignon grapes are known to have a strong taste of dark fruits such as strawberries, cherries and blackberries with aromas of spices such as vanilla and cinnamon. The wines from Cabernet Sauvignon are also characterized by their rich body and strong tannins, which give them a dry and persistent taste (Voinesco et al., 2023).

The purpose of the research reflected in this article consists in:

- studying the current state and prospects for the development Cabernet sauvignon variety in the Codru Wine Region of the Republic of Moldova (clones, technology, vineyards areas, yield, quality of grapes and wine etc.).

MATERIALS AND METHODS

The studies reflected in this article were carried out on different clones of the Cabernet Sauvignon variety grown in different wine-growing regions (Stefan Vodă, Gagauzia, Anenii Noi, Hâncești, Ialoveni etc.) during the years 2017-2022.

Also, the following databases and information were used:

- National Bureau of Statistics (NBS) of the Republic of Moldova;
- National Office of Vine and Wine (NOVW) etc.

MS Office Excel (2019) was used for the mathematical processing of the data.

RESULTS AND DISCUSSIONS

The wine register of the Republic of Moldova was approved and implemented by HG 292/2017.

According to the GD, the Wine Register is a departmental register and is part of the state registers. The wine register represents a systematized totality of data on wine plots with an area of more than 0.15 ha, on wine units and on the traceability of wine products.

In the Republic of Moldova, in according to the Catalog of Plant Varieties for the 2023 year, are permitted the following clones of the Cabernet Sauvignon variety are grown - R-5, ISV F-5, 07, 15, E-153, 169, 191, 216, 337, 338, 341, 685 (Machidon et al., 2023; Voinesco et al., 2023).

The clone 169 of Cabernet Sauvignon variety, is a French clone. The clone 169 has good berry size, high vigour, and a well-balanced acid to sugar ratio. The wine can be higher in alcohol content depending on the winemaking technology and style. Clone 169 of Cabernet Sauvignon variety was created to produce a big and bold Cabernet wine with intense complexity. The color is a vibrant, deep red. The mouth feel tends to be bold and linger. This grape is definitely for the adventurous winemakers.

The clone 337 of Cabernet Sauvignon variety is best known as one of the premier French clones. The clone 337 gives good yield, the clusters with small intense berries, and very fruit forward flavors. The wines from the clone 337 create a lush mouthfeel, they have deep color, and intense dark fruit tasty, chocolate, and intense tannins. Smooth, fine-grained tannins coat the palate, where the more opulent character of this clone really comes out, reducing the impression of Cabernet Sauvignon typicity.

The clone 191 of Cabernet Sauvignon variety is best known as one of the premier French clones. The clone 191 gives low level of yield, the clusters with small intense berries. Fertility is low to medium. The vigour of vines is medium. Sugar content in berries is high, but acidity is medium. Potential colour and tannic structure of wines are high.

Table 1. Distribution of areas with Cabernet Sauvignon variety according to administrative districts

No.	District	Vineyard's areas, ha	The share of districts in the total area of the variety, %
1	Anenii Noi	293.67	5.86
2	Basarabeasca	116.12	2.32
3	Briceni	0.00	0.00
4	Cahul	859.63	17.14
5	Cantemir	336.62	6.71
6	Călărași	3.63	0.07
7	Căușeni	230.49	4.60
8	Cimișlia	167.84	3.35
9	Criuleni	12.50	0.25
10	Dondușeni	0.00	0.00
11	Drochia	0.00	0.00
12	Dubăsari	0.00	0.00
13	Edineț	0.00	0.00
14	Fălești	0.00	0.00
15	Florești	0.49	0.01
16	Glodeni	0.00	0.00
17	Hîncești	256.41	5.11
18	Ialoveni	43.08	0.86
19	Leova	277.81	5.54
20	Nisporeni	2.78	0.06
21	Ocnița	0.00	0.00
22	Orhei	5.54	0.11
23	Rezina	0.00	0.00
24	Rîșcani	0.00	0.00
25	Sîngerei	0.00	0.00
26	Soroca	0.00	0.00

No.	District	Vineyard's areas, ha	The share of districts in the total area of the variety, %
27	Strășeni	54.65	1.09
28	Șoldănești	0.00	0.00
29	Ștefan Vodă	716.07	14.28
30	Taraclia	626.75	12.50
31	Telenești	6.42	0.13
32	Ungheni	0.60	0.01
33	UTA Găgăuzia	906.85	18.08
34	Mun. Bălți	0.00	0.00
35	Mun. Chișinău	29.03	0.58
36	Mun. Tiraspol	67.63	1.35
		5014.62	100.00

Source: NBS & NOVW, processed by authors

The Cabernet Sauvignon variety according to the wine register of the Republic of Moldova occupies 100 ha, which are distributed different in the wine regions.

The vineyards area occupied by the Cabernet Sauvignon variety in the Codru grape and wine region is 1154.45 ha, or 23.02%. The largest area of vineyards with the Cabernet Sauvignon variety is the Leova, Hincesti, Anenii Noi districts (Table 1, Figure 1).

The area occupied by the Cabernet Sauvignon variety in the Stefan Voda grape and wine region is 1014.19 ha or 22.22%. The largest area of vineyards with the Cabernet Sauvignon variety is the Ștefan Vodă, Căușeni districts (Table 1, Figure 1).

The area occupied by the Cabernet Sauvignon variety in the Valul lui Traian grape and wine region is 2845.97 ha or 56.75% The largest area of vineyards with the Cabernet Sauvignon variety is the Taraclia, Gagauzia, Cahul districts (Table 1, Figure 1).

In the reference years, an increased harvest potential was found in Clone 169 and Clone 337, a lower harvest potential in Clone 191.

Fertility was higher in clone 169 and clone 191 compared to clone 337.

As a result of the statistical processing of the Cabernet Sauvignon harvest data according to the meteorological conditions of the year through the correlation and regression analysis method, the linear regression equations were obtained for each year separately and as a whole (Table 2).

The regression characteristics for the linear regression equations reflected in Table 2 are reflected in Table 3.

The average correlation coefficient of 0.42 proves the existence of a low positive correlation, with an influence level of 18% and the error value - 0.19 (Table 3).

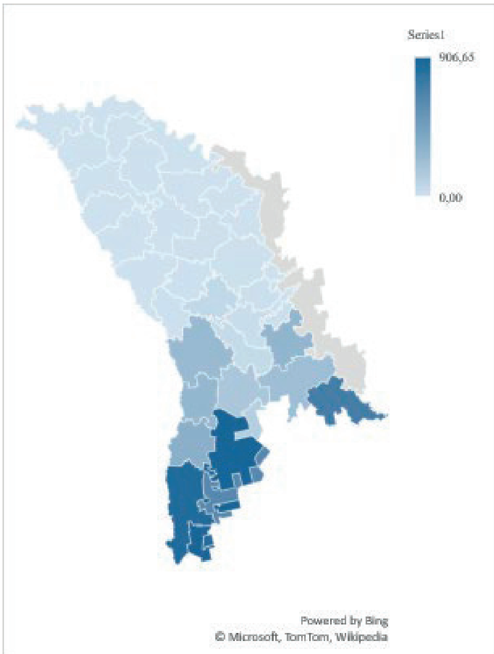

Figure 1. The map of distribution of areas with Cabernet Sauvignon variety according to administrative districts (Source: NBS & NOVW, processed by authors)

Table 2. Linear regression equation (y = a + bx) between the sum of active temperatures and the harvest of Cabernet Sauvignon grapes in dependence of year meteorological condition Characteristics of regression analysis

Year	Linear regression equation (y = a + bx)
2017	y = -0.0055 + 1.8198x
2018	y = 0.0053 + 1.6925x
2019	y = 0.0009 + 1.7371x
2020	y = -0.004 + 1.3235x
2021	y = -1.3239 + 1.3239x
2022	y = -0.0057 + 1.8885x
average	y = -0.0022 + 1.6205x

Source: Processed by authors

The sugar content registered a higher level in clone 169 and clone 191 compared to clone 337. As a result of the statistical processing of the Cabernet Sauvignon sugar content data according to the medium temperature through the correlation and regression analysis method, the linear regression equations were obtained for each year separately and as a whole (Table 3, Figure 2).

Table 3. Characteristics of regression analysis for dependence between the sum of active temperatures and the harvest of Cabernet Sauvignon grapes

Year	Correlation coefficient	Determination coefficient (the square of the correlation coefficient)	Error
	r	$d_{yx}=r^2$	Sr
2017	0.44	0.19	0.19
2018	-0.22	0.0	0.21
2019	0.22	0.05	0.21
2020	0.41	0.18	0.20
2021	0.38	0.18	0.19
2022	0.31	0.17	0.23
average	0.42	0.18	0.19

Source: Processed by authors

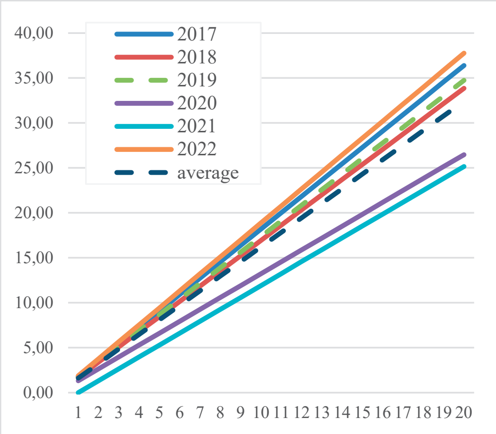

Figure 2. The correlative dependence between the sum of active temperatures and the harvest of Cabernet Sauvignon grapes (Source: Processed by authors)

The regression characteristics for the linear regression equations reflected in Table 4 are reflected in Table 5.

The average correlation coefficient of 0.73 proves the existence of a high positive correlation, with an influence level of 53% and the error value - 0.15 (Table 5, Figure 3).

Table 4. Linear regression equation (y = a + bx) between the medium temperature and the sugar content of Cabernet Sauvignon grapes in dependence of year meteorological condition

Year	Linear regression equation (y = a + bx)
2017	y = -0.0102+22.932x
2018	y = -0.0022+21.805x
2019	y = -0.0011+21.4964x
2020	y = -0.0139+24.0404x
2021	y = -0.0095+21.2859x
2022	y = -0.0092+20.6946x
average	y = -0.0076+22.0423x

Source: Processed by authors

Table 5. Characteristics of regression analysis for dependence between the medium temperature and the sugar content of Cabernet Sauvignon grapes

Year	Correlation coefficient	Determination coefficient (the square of the correlation coefficient)	Error
	r	$d_{yx}=r^2$	Sr
2017	0.75	0.56	0.14
2018	0.58	0.34	0.17
2019	0.48	0.23	0.19
2020	0.51	0.26	0.18
2021	0.74	0.55	0.14
2022	0.68	0.46	0.15
average	0.73	0.53	0.15

Source: Processed by authors

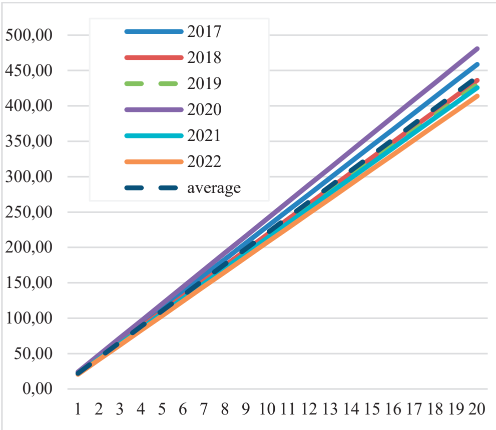

Figure 3. The correlative dependence between the medium temperature and the sugar content of Cabernet Sauvignon grapes (Source: Processed by authors)

CONCLUSIONS

The Cabernet Sauvignon variety is the most widespread wine variety in the Republic of Moldova.

Most vineyards are established with planting material imported from nurseries in Italy, France, etc.

The largest area of vineyards with the Cabernet Sauvignon variety is the Ștefan Vodă, Căușeni districts (Ștefan Vodă Region); Leova, Hincesti, Anenii Noi districts (Codru Region) and Taraclia, Gagauzia, Cahul districts (Valul lui Traian Region)

In the reference years, an increased harvest potential was found in Clone 169 and Clone 337, a lower harvest potential in Clone 191.

Fertility was higher in clone 169 and clone 191 compared to clone 337.

The sugar content registered a higher level in clone 169 and clone 191 compared to clone 337.

The average correlation coefficient of 0.42 proves the existence of a low positive correlation, with an influence level of 18% and the error value – 0.19.

The average correlation coefficient of 0.73 proves the existence of a high positive correlation, with an influence level of 53% and the error value – 0.15.

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THE INFLUENCE OF VITICULTURAL PRACTICES ON THE BERRY COMPOSITION OF MERLOT VARIETY GROWN IN THE WEST OF ROMANIA CLIMATE

Eleonora NISTOR[1], Alina DOBREI[1], Giovanni MATTI[2], Alin DOBREI[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, Department of Horticulture, Calea Aradului 119, 300645, Timisoara, Romania
[2]Universita degli Studi Firenze, Dipartimento Di Scienze Produzioni Agroalimentari E Dell'ambiente, Viale Delle Idee, 30, 50019, Sesto Fiorentino, Italy

Correspondent author emails: ghitaalina@yahoo.com, alin1969tmro@yahoo.com

Abstract

Recent climatic conditions have favoured the expansion of red varieties in previously unsuitable areas for high-quality winemaking. The high degree of temperature variability during the growing season makes it challenging for berries to fully ripen in time for harvest. The objective of this research was to improve the berry quality and sensory characteristics of the Merlot variety in the Recaş vineyards area by implementing various viticultural practices. The timing and intensity of leaf removal and cluster thinning were examined in experiments carried out in 2020 and 2021. The cluster thinning had a minimal effect on basic berry chemistry at harvest in 2020 but increased pruning weight and cluster weight. Vine vigour and essential berry chemistry were not strongly influenced by clusters thinning in 2021. The level of titratable acidity was lower after leaf removal, but other essential quality indicators remained unchanged. Viticultural practises not only increased the berry quality but also the anthocyanin content of berries.

Key words: *berry quality, cluster thinning, leaf removal, sugars, titratable acidity.*

INTRODUCTION

Grape cultivation is one of the most important agricultural activities on the planet. According to FAO statistics (2021), the global area cultivated with grapevines in 2021 was 6.73 million hectares, with 73.5 tonnes of grapes for wine and table, resulting in 755 352 081 hl of wine. The world's largest grape production is concentrated in four countries: Italy, Spain, France, and China (FAOStat, 2021). Grapevine cultivation is restricted by climate regions, with annual isotherms ranging from 10°C to 20°C, with the possibility of expansion in favourable microclimates (Kosik et al., 2017). Different viticultural practises can compensate for less-than-ideal conditions for Merlot grapes, which do not reach full maturity every year (Anić et al., 2021). To achieve the best quality of the grapes and the wine, winemakers must understand how to fully ripen this variety in order to prevent unpleasant, grassy aromas (Trujillo et al., 2022). The process of berry growth and development occurs through cell division in the first stage; the second stage is the veraison stage, during which the accumulation of sugars in the pulp and skins, of anthocyanins, and potassium begins and towards the end, the colouring and softening of the berries; the third stage is the ripening stage, during which sugars accumulate primarily in the pulp and anthocyanins in the skins, especially in red varieties (CooMbe & McCarthy, 2000; Nan et al., 2019). A balance between vegetative and productive growth is required for optimal compound accumulation in berries (Tomasi et al., 2020). The crop load (the ratio of grape yield and the weight of the pruning wood) is accepted as an indicator of grapevine balance, with optimal values ranging from 5 to 10 (Silvestroni et al., 2019). Another useful tool for assessing vine balance is the ratio of yield to leaf area (expressed in cm^2/kg or m^2/kg), with leaf photosynthesis activating the production of carbohydrates that are transported to the berries (Del Zozzo et al., 2022). The management system, in addition to the vigour of the variety, influences photosynthesis efficiency and, indirectly, crop load (Nistor et al., 2021). A crop load that is too high (over 15 clusters) restrains the proper berry quality achieved at harvest, and a crop load that is too low (less than five bunches) is not economically efficient (Candar et al., 2020). Sucrose is transported to the berries after photosynthesis in the leaves, where sugars measured as total soluble solids (TSS) accumulate between 18.5 and 27ºBrix, depending on variety, viticultural practises, and environmental conditions (Lu et al., 2022). According to some studies, thinning leaves or bunches reduced production and increased sugar content compared to vineyards with high grape production (Sivilotti et al., 2020), but other studies show that these practises had little to no effect on the amount of sugar that accumulated in berries up until harvest (Williams et al., 2023). After fermentation, grape seed compounds such as sugars, organic acids, polyphenols, and aromas significantly influence the wine's final quality (Mesić et al., 2020).

The study therefore concentrated on achieving the best Merlot grape quality (sugar, anthocyanins, pH, TA) by adjusting the crop load, leaf removal and cluster thinning at various growth stages during the 2020 and 2021 growing seasons.

MATERIALS AND METHODS

Plant materials

The Merlot (grafted on Kober 5 BB rootstocks) plots were planted in the Recaș vineyards in 2009 and were used for research in 2020 and 2021 growing seasons. The Recaș vineyards are located 24 kilometres from Timișoara, at latitude 45°48'4.18"N and longitude 21°30'42.89"E. The Recaș vineyards cover an area of more than 1000 hectares spread out over hills with calcareous or clay soils.

The experimental plots had 10 rows and 5 vines per row with a simple Guyot training, a planting distance of 2.2 m between rows and 1 m between vines per row, and an average density of 4,545 vines/hectare. During dry pruning, 45 buds per vine were retained. The vigorous shoots were hedged during the growing season when their tips reached a height of 30 cm above the highest trellis. During the growing season, pest and disease control measures were implemented based on past performance, alerts, and weather conditions. The Timișoara meteorological station and satellite data for the Recaș location (https://freemeteo.ro/vremea/ recas/) were used to determine the monthly precipitation and daily temperature during the growing season.

Field experiment

The vines in the experiment were divided into eight blocks of five vines each, for a total of 40 vines. Blocks were randomly assigned to the fruit set, pre-veraison, veraison, and before ripening stages. The fruiting load was set at 15, 30, and 45 bunches per vine; the first four basal leaves on the shoot were removed to expose the clusters, as Merlot is a medium-vigorous variety; apical clusters that remained less developed were removed. Leaf and bunch thinning were done simultaneously.

Measurement of shoot growth

At flowering, the total number of shoots was counted, of which six were marked for length monitoring (cm/day) every seven days, beginning from the second week of June until veraison in the last week of July, using a measuring tape. The shoot growth measurements were useful for estimating daily growth, correlated with phenological stages and the effects of crop load and leaf removal on their length.

Cluster and berry sampling

From fruit set to ripening, 50 berries were collected from each selected vine at 14-day intervals. The samples were placed in labelled plastic bags and transported to the laboratory in a freezing bag, where they were kept at -20ºC for future analysis. The total weight of bunches on a vine, the average weight of a cluster, and the average weight of berries in a cluster were all recorded.

Clusters on marked shoots were collected separately, and berries were measured for sugars, titratable acidity, and pH. Before analysis, the berries were defrosted and crushed, and the naturally drained juice was decanted into 50 ml cylinders. The sugar content of the must was determined using a Brix HI96813 digital refractometer (Hanna Instruments, Inc). Total acidity (TA H_2SO_4) and pH were measured with HI 84532 titrator (Hanna Instruments, Inc).

Statistical Analysis

Analyses of variance (ANOVA) for yield and berry components were performed using GraphPadPrism Software, Version 8.4.2 (San Diego, California, USA, 2020) and XLStat (by Addinsoft, 2018, Statistical and Data Analysis Solution Version 2018.7.5). Comparisons were made by the LSD test with significance levels of 0.05.

RESULTS AND DISCUSSIONS

Climate conditions

Following a less rainy spring in 2020 (Figure 1), there was abundant precipitation (over 100 mm) during the shoot growing stage (June, July) until veraison, with the phenological stages developing at a constant rate. There was much less rain and much higher temperatures during the same time period in 2021. August was the warmest and driest month, following veraison in both growing seasons. It was relatively wet in 2020 during grape ripening, which began in the second decade of September, but very dry in 2021.

The maximum air temperature reached more than 35°C during the summer of 2021, but no severe sunburn damage to grape berries was observed.

Shoot length growth was unaffected by leaf and bunch thinning. Regardless of the fruiting load, the lateral bunches were the most exposed to the sun after the leaves were removed.

Vegetative growth in 2020-2021

Leaves or clusters thinning had no effect on the length of the shoots, but the earlier these treatments were done, the greater the impact on the growth rate of the shoots (Figures 2 and 3).

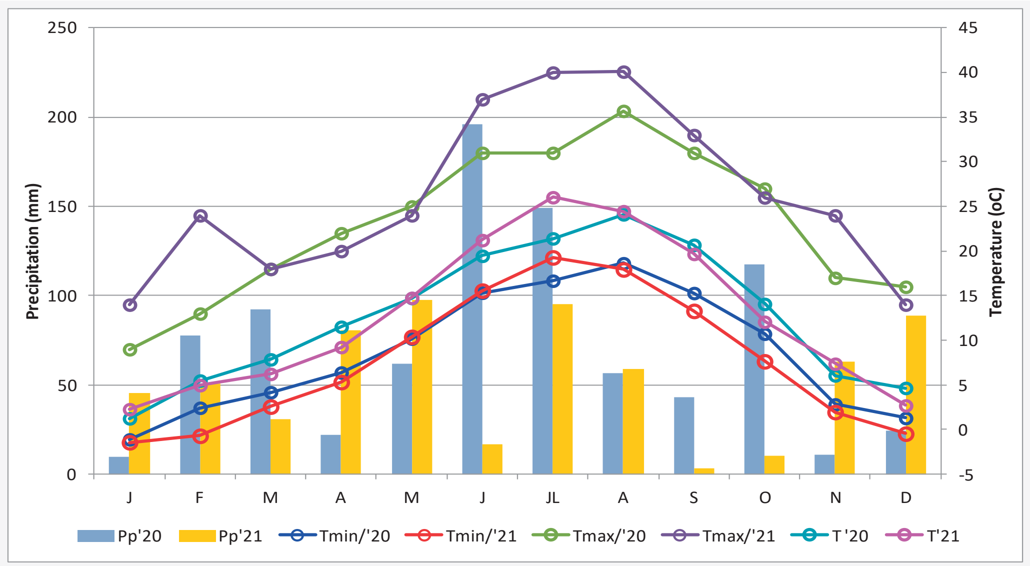

Figure 1. Temperature and precipitation during 2020-2021
(Pp - precipitation; Tmin - minimum temperature; Tmax - maximum temperature; T - average temperature)

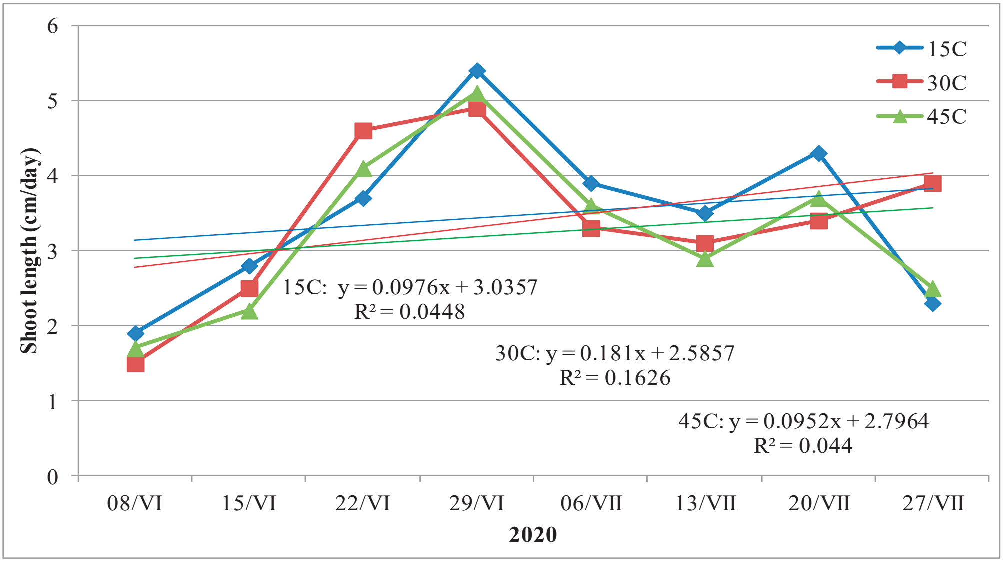

Figure 2. Shoot growth (cm/day) from the beginning of June to the end of July in 2020 (15°C, 30°C and 45°C are abbreviations for crop load on vine; data were collected from vines selected for leaf removal and cluster thinning)

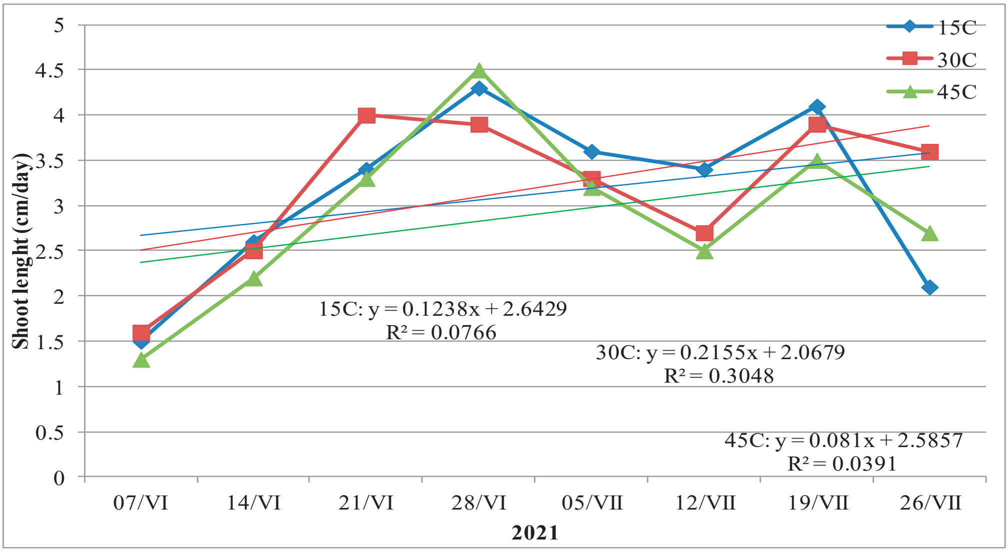

Figure 3. Shoot growth (cm/day) from the beginning of June to the end of July in 2021 (15°C, 30°C and 45°C are abbreviations for crop load on vine; data were collected from vines selected for leaf removal and cluster thinning)

With the exception of the crop load of 30 clusters in 2021, which positively influenced shoot length growth, cluster exposure had no effect on shoot growth rate.

Grape production components

Cluster thinning during berries setting, with a crop load of 15 clusters, significantly decreased grape production per vine (Tables 1 and 2).

Table 1. The influence of basal leaf removal and cluster thinning on crop yield (2020)

Crop load	Yield (kg/vine)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	1.19^{b}	1.29^{b}	1.59^{b}	1.59^{b}
30 clusters	3.40^{a}	2.65^{a}	3.30^{a}	3.21^{a}
45 clusters	2.43^{a}	3.20^{a}	4.65^{a}	4.98^{a}

$^{a, b}$ Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

However, there was no significant difference in the yield after leaf thinning at 30 and 45 clusters of crop load. During the 2021 growing season, only the 45 cluster crop load per vine showed significant yield differences when cluster thinning was done before, during or after veraison.

Table 2. The influence of basal leaf removal and cluster thinning on crop yield (2021)

Crop load	Yield (kg/vine)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	1.27^{b}	1.56^{b}	1.65^{c}	1.53^{b}
30 clusters	3.52^{a}	2.71^{ab}	3.42^{b}	3.15^{b}
45 clusters	2.94^{a}	3.38^{a}	4.80^{a}	4.45^{a}

$^{a, b, c}$ Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

Leaf and severe cluster thinning (crop load of 15 clusters) at berries setting significantly decreased grape yield in the 2021 growing season (Table 2) compared to a crop load of 45 clusters per vine. In the berry setting stage, grape yield did not differ significantly between 30 and 45 clusters of crop load. When leaf and bunch thinning were performed at veraison, grape production differed for all three crop loads, with the higher number of clusters per vine yielding the most. Regarding cluster weight, the interaction between the three factors - leaf removal, cluster thinning, and cluster weight respectively was significant.

Table 3. The influence of basal leaf removal and cluster thinning on cluster weight (2020)

Crop load	Cluster weight (g)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	89^{a}	101^{a}	122^{a}	105^{a}
30 clusters	76^{c}	81^{b}	93^{b}	80^{b}
45 clusters	59^{c}	68^{c}	76^{c}	86^{b}

$^{a, b, c}$ Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

The lower number of clusters per vine obviously resulted in larger and heavier clusters when compared to the other two crop loads of 30 and 45 clusters per vine. However, cluster weight increased only for 15 crop loads when cluster thinning was done before and after veraison (Table 3). Regardless of crop load, the difference in cluster weight at berry set was insignificant. In fact, when analyzing bunch weight for the four phenological phases, no significant differences were found as a result of crop load influence.

Heavier clusters were also observed in the 2021 growing season at 15 and 45 clusters per vine (Table 4). The smallest weight differences between bunches, regardless of crop load, were when leaf removal and bunch thinning were done at veraison; the differences were insignificant at other growing stages.

Table 4. The influence of basal leaf removal and cluster thinning on cluster weight (2021)

Crop load	Cluster weight (g)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	96^{a}	106^{a}	115$^{a, b}$	108^{a}
30 clusters	68^{b}	74^{b}	124^{a}	96^{ab}
45 clusters	86^{a}	84^{b}	106^{ab}	78^{b}

$^{a, b}$Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

In the 2020 growing season, no significant differences in sugar accumulation (ºBrix) were found for the levels of the cluster thinning (Table 5). The effect of leaf removal on sugar accumulation was more obvious when the treatments were done at veraison, and the berries were exposed to sunlight before ripening began.

Table 5. The influence of leaf removal and cluster thinning on TSS (ºBrix) (2020)

Treatment	TSS (°Brix)			
	Berry set	Pre-veraison	Veraison	Before ripening
Leaf removal	15.3[a]	18.4[a]	20.8[a]	21.9[b]
Cluster thinning	16.9[a]	19.1[a]	21.5[a]	22.6[a]

[a, b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

Table 6. The influence of leaf removal and cluster thinning on TSS (ºBrix) (2021)

Treatment	TSS (°Brix)			
	Berry set	Pre-veraison	Veraison	Before ripening
Leaf removal	16.5[b]	19.6[a]	21.4[a]	22.9[b]
Cluster thinning	17.1[a]	20.4[a]	22.3[a]	23.6[a]

[a,b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

When grape berries were exposed to sunlight through leaf removal and cluster thinning during the same phenological phase in the 2021 growing season, the differences in sugar accumulation were insignificant (Table 6). However, when the treatments were applied at different phenological stages, there were significant differences in sugar accumulation, taking into account that sugar accumulation increases as the berries reach maturity.

Table 7. Berry components depending of growing season timing of leaf removal and cluster thinning

Berry components	Berry set	Pre-veraison	Veraison	Before ripening
	Treatment during 2020 growing season			
pH	3.4[a]	3.6[a]	3.8[a]	3.8[a]
TA (g/L H_2S0_4)	5.5[a]	4.9[a]	4.7[a]	4.3[a]
Anthocyanins (mg/g)	0.71[a]	0.69[a]	0.63[a]	0.72[a]
	Treatment during 2021 growing season			
pH	3.2[a]	3.5[a]	3.3[a]	3.5[a]
TA (g/L H_2S0_4)	5.7[a]	5.0[a]	4.9[a]	4.5[a]
Anthocyanins (mg/g)	0.73[a]	0.68[a]	0.65[a]	0.76[a]

[a, b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

In both growing seasons, except for sugars, no significant relationships were found between treatment timing and the other components of berry juice (TA, pH, and anthocyanins) as a result of grape berry exposure to sunlight after leaf removal and cluster thinning (Table 7).

It was difficult to find a correlation between treatments (leaf removal, cluster thinning, and treatment timing). Furthermore, there was a lot of rain, and it was very hot in June and July 2020, which stimulated the growth of the shoots and made measurements harder to manage. Grape yield increased, however, when the crop load per vine was 15 or 30 clusters. When the treatments were applied at berry set, the production increased for the higher crop load of 45 clusters. When the treatments were applied early in the growing season, at berry set, pre-veraison, or veraison but not before ripening, bunch weight increased at a low crop load (15 clusters). The weight of the berries in the cluster increased as a result of leaf removal and cluster thinning, which improved grape yield. Early leaf removal (at berry set) affected berries by sunlight and, consequently, sugar accumulation; however, during both growing seasons, key berry juice constituents (pH, TA, and anthocyanins) were not significantly influenced by the timing of treatments and crop load.

Leaf removal when the temperature was very high at the end of July and especially in August was beneficial for reducing the malic acid content of berry juice (Ghiglieno et al., 2020); sunlight and temperature were also important for anthocyanin accumulation (Tarricone et al., 2020). The lowering of crop load, or vine vigour, had no noticeable impact on berry ripening or on the main components of the berries, such as sugars which could explain the absence of significant differences between the chemical components of berry juice. The berry content in various qualitative components influenced not only the amount of sugar but also the grape yield. The anthocyanin content was influenced by the interaction of treatments (leaf removal and cluster thinning) as well as the increase in temperature at the cluster level as a result of leaf removal (Alba et al., 2022). Previous research found that when the crop load is balanced with the canopy, the accumulation of sugars or other qualitative components from berries is unaffected in hot climates (Anić et al., 2021; King et al., 2012; Pieri, 2010).

Sugar accumulation was relatively stable despite differences in humidity between the two growing seasons, indicating that viticultural practises had a relatively low influence on this qualitative component compared to anthocyanin content (O'Brien et al., 2021; de Rosas et al., 2022).

However, contradictory findings were found regarding sugar accumulation, which was influenced by viticultural practises and environmental conditions (Navrátilová et al., 2020; Aru et al., 2022); TSS content was found to be higher after the cluster thinning or leaf removal in some studies (Chorti et al., 2010; Wang et al., 2022). Usually, the presence of more sugar has always been associated with ripened berries, serving as an indicator of berry maturity (Gris et al., 2010; Previtali et al., 2021).

Coarfă & Popa (2020) found an evolution of 38 g of sugar content over a period of 13 days, from 187.5 to 225.8 g/L, during the gradual harvesting of Merlot berries from the final decade of August to the second decade of September 2017 (both months with rainfall deficiency). In a similar climate (temperature and rainfall) from July to September (2020), Onache et al., (2021) found that Merlot grapes ripened approximately 10 days earlier than the previous year 2019 due to the highest temperatures recorded during the growing season, with 210 g/L sugar content, 3.06 total acidity (H_2SO_4), and a lower anthocyanin content of 464.87 mg/L.

CONCLUSIONS

The research in 2020 and 2021 were quite similar, with an emphasis on the effect of crop load, leaf removal, and grape berry exposure to sunlight on Merlot grapes. Similar outcomes were observed in both seasons for grape yield and berry quality components, with minor differences influenced by precipitation during the pre-veraison and veraison time frames. Small grape yield differences were also observed between the treatments, especially between the grape yield after leaf removal and after the cluster thinning.

In comparison to 2020, however, the grapevine has been less affected by leaf removal and cluster thinning treatments in 2021. The impact of these treatments on shoots growth has been negligible. The research results from both growing seasons show that cluster thinning had little effect on grape yield and berry components in the 2021 growing season. When the number of clusters was reduced in the berry set or pre-veraison stages, the vines had higher vigour and larger clusters as a result of the climatic conditions of the 2020 growing season. Finally, grapevine balance is determined by the canopy vigour of the grape variety and the timing of leaf removal, both of which can influence grape berry quality improvement.

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THE ACIDITY IN THE MUST OF THE FETEASCĂ ALBĂ VARIETY, IN RELATION TO FERTILIZATION AND THE CONTENT OF MACROELEMENTS IN THE PLANT

Luminiţa Nicoleta PARASCHIV, Lucian RĂUS, Diana Elena BOLOHAN, Elena-Liliana CHELARIU, Bogdan Vlad AVARVAREI, Mariana VOLF

Iaşi University of Life Sciences "Ion Ionescu de la Brad",
Mihail Sadoveanu Alley, No. 3, Iasi, Romania

Corresponding author email: mariana.volf@uaiasi.ro

Abstract

The research focused on the analysis of the impact of these macroelements, used in different doses and ratios, on the accumulations in the plant and on the production quality, respectively on the varied acidity of Fetească albă must. The analysed Fetească albă variety is one of the predominant varieties of the Sorogari wine-growing center, belonging to the Iasi vineyard. The soils of the area under study are part of the group of anthropogenic soils, such as cambic cherno-zones, forest ash and regosols, with uneven fertility. Values of the content in the plant of 2.12% N, 0.183% P, 0.883 K as well as acidity values of 5.27 g/l H2SO4, by applying fertilizers in the minimum necessary doses, certify that they can be at the level statistical assurances It can be stated that the use of doses of fertilizers according to the criterion of sufficiency, in the analysed agro-eco-pedological context, ensures that the optimal experimental doses, optimal contents in the plant of macronutrients and implicitly, the nutrition environment is protected from pollution.

Key words*: foliar diagnosis, level of sufficiency, nutritional balance.*

INTRODUCTION

Primary macroelements are essential for the nutrition of cultivated plants and, when they are in optimal quantities, they compete in obtaining the expected harvests, of a high level and of a superior quality. Their presence in the soil and their translocation in plants, through root or extra-root absorption processes, make possible the development of numerous and complex chemical, biochemical and physiological processes, which lead to a sustained metabolic activity. Any nutritional imbalance has its causality in a dysfunction of these processes, induced in turn, primarily by the nutritional environment - the soil - but also by a series of other factors, with equal importance, such as the climate, the anthropic factor, etc. Fertilization in different doses and ratios of fertilizers improves the yield but also the quality of crops, establishing important correlations in the soil-plant relationship (Habibur et al., 2021).

In relation to the expected production and the variety, the vine has a nitrogen, phosphorus and potassium consumption per ton of grapes that oscillates between 6-15 kg N, 1-3.7 kg P_2O_{55}, 3.8 and 15 kg $K_{22}O$ (Davidescu & Davidescu, 1992). The need for such quantities resides in the role of these elements in the general metabolism of this plant. The plastic role of nitrogen is well known, in the composition of biological membranes, through structural and enzymatic proteins, which also intervene in transport (Mengel, 1987).

Among its multiple functions, phosphorus is highlighted by its fundamental role in the storage and transmission of genetic information. Phosphorus has implications in energy metabolism and photosynthesis, in different phenophases of plant growth. (Yan et al., 2021). A balanced nutrition in phosphorus increases the resistance of plants to drought and frost, shortens certain stages of vegetation in organogenesis, but also compensates, through rebalancing, the excess of nitrogen.

Potassium is the most important cation in the life of plants, its functions being multiple such as: cell division, growth of meristematic tissues, absorption of nutrients, synthesis and activation of enzymes, role in photosynthesis, in carbohydrate and protein metabolism

(Hassanein et al., 2021). In the cellular structure of plants, approximately 10-20% of total potassium is found in chloroplasts and another 10-20% in the cytoplasm (Marschner, 1993).
The study undertaken refers to the content and variation of these essential elements in the plant material, in the vine, in relation to the administration of fertilizers, in different doses and ratios, precisely to stimulate these processes, which tend to be reflected in the quantity but also quality of grape production. The central goal of the research is to bring information about the use of small doses of fertilizers applied on the criterion of sufficiency, protecting the environmental and nutritional environment, possible to use to obtain the organoleptic properties specific to obtaining wines of superior quality, with designation of origin.

MATERIALS AND METHODS

The research took place in three consecutive years, in a vineyard, belonging to the Sorogari viticultural center in the Iasi vineyard. This territory, which currently belongs to a family association, falls geographically in the Jijia-Bahlui depression, being located in its southern part.
In general, in this vineyard, ripening conditions are ensured for grapes from the I-V ripening periods and sometimes for those with late and very late ripening, a fact demonstrated by the value of the global heat balance, which stands for a multi-year period at the value of 3237.0°C.
The soil present in the area of the experimental polygon is a mesocalcareous cambic chernozem (weakly leached or decarbonated), loamy-clay, developed on loessoid deposits, uncovered, unirrigated. The determined agrochemical properties of the soil unit studied were: pH (H_2O): 6.9-7.5, neutral to slightly alkaline reaction, medium humus content (2.4-2.5% H), a low to medium content in nitrates (1.9-3.9 mg NO_3/100 g soil), low content in mobile phosphorus (20.8-52.8 ppm P-AL); good supply status in mobile potassium (260-301 ppm K-AL). The content of 17.0 me % Ca^{2+}, indicates a high fertility. Among the exchangeable cations, the content of 2.06-2.88 me % Mg^{2+} places the soil in a medium fertility class. Boron is at the lower limit of a medium insurance (0.4-0.5 ppm B), copper at values > 25 ppm indicates an excess supply of the soil, mobile zinc at high levels respectively 1.8-2.0 ppm Zn.
The biological material used was the Feteasca Alba vine variety, grafted on the Kober 5 BB rootstock, derived by clonal selection from the population of Berlandieri X Riparia Teleky 5 A.
During the experimentation two factors were implemented and analysed independently such as **dose levels** and **fertilizer combinations** as a result of the combination of fertilizing elements (Table 1).

Table 1. Factors implemented during the experimentation

Factor 1 - Dose levels			
Dose levels	**N, kg/ha**	**P, kg/ha**	**K, kg/ha**
D1 - level 0.5	50	25	90
D2 - level 1.0	100	50	180
D3 - level 1.5	150	75	270
Factor 2 - Fertilizer combinations			
Fertilizer combinations	**N**	**P**	**K**
Control	N	P	K
T1	N	-	K
T2	N	P	-
T3	-	P	K
T4	N	-	-
T5	-	P	-
T6	-	-	K

The 0.5 dose level represents the minimum administered doses, respectively the state of sufficiency, the 1 dose level is represented by optimal dose and the 1.5 dose level being studied in research, to track the agronomic and economic efficiency of the maximum doses.
The combination of fertilizing elements was introduced for the study of the singular or combined effect, on the aspects addressed, since the relationships of antagonism and synergism between the essential elements on the one hand and between them and the rest of the elements in the soil solution and at the level of the various vegetative organs are already known.
For the analysis of plant samples, methods currently used in foliar diagnosis were used:
- dosing of total nitrogen, mineralization option with sulfuric acid, distillation and titration of ammonia with H_2SO_{44}; SR ISO 11261:2000

- total phosphorus dosage - wet mineralization, with ammonium molybdate and reduction with stannous chloride, colorimetric dosage SR EN 16192:2012
- dosing of total potassium, by mineralization with a mixture of sulfuric acid and perchloric acid and dosing by flame photometry. STAS 7184/7-87

The harvesting of leaves, for the foliar diagnosis, was done in the flowering phenophase.

The chemical analyzes of the must consisted in determining the total acidity, by titration with a 0.1 n NaOH solution ; SR EN 16192:2012

RESULTS AND DISCUSSIONS

The analysis of the total nitrogen content in the leaves (Table 2), reveals its values from 2.12 to - D1 to 2.22% to D3, values statistically different from significant to very significant and included in supply levels from weak at optimum.

Table 2. The influence of the fertilizer dose on the evolution of Nt (%) in the leaves of Fetească albă variety

Dose level		N_t (%)	% increase	Differences
Control	0	2.05	100.0	-
D1	0.5	2.12	103.5	0.07*
D2	1	2.17	105.9	0.12**
D3	1.5	2.22	108.1	0.16***
***LSD**	5 %	0.06		
****LSD**	1 %	0.10		
*****LSD**	0.1%	0.14		

LSD - least significant difference

The Nt values in the leaves in relation to the combination of fertilizing elements (Table 3), oscillate from the NPK variant (control), where optimal values of 2.27% and only 2.06% are recorded for fertilizing exclusively with phosphorus or potassium (T5 and T6). As expected, the binary fertilized variants containing nitrogen, NK (T1) and NP (T2), obtained similarly results as control.

At flowering (Table 4), the 0.5 dose level (D1) ensures in the leaves a total phosphorus value of 0.183% Pt, higher than the control by 0.014% Pt (statistically significant), very close to the optimal content for this phenophase (0,19-0.25% Pt) but below the level of using the optimal doses (D2).

Table 3. The influence of the fertilizer combinations on the evolution of Nt (%) in leaves of Fetească albă variety

Fertilizer combinations		N_t (%)	% increase	Differences
Control	NPK	2.27	100.0	-
T1	NK	2.20	96.7	-0.07
T2	NP	2.19	96.5	-0.07
T3	PK	2.06	90.6	-0.21
T4	N	2.15	94.9	-0.11
T5	P	2.06	90.5	-0.21
T6	K	2.06	90.7	-0.21
***LSD**	5 %	0.05		
****LSD**	1 %	0.07		
*****LSD**	0.1 %	0.09		

LSD - least significant difference

Table 4. The influence of the fertilizer dose on the evolution of Pt (%) in the leaves of Fetească albă variety

Dose level		P_t (%)	% increase	Differences
Control	0	0.169	100.0	-
D1	0.5	0.183	108.6	0.014*
D2	1	0.196	116.2	0.027**
D3	1.5	0.210	124.6	0.041***
*LSD	5 %	0.014		
**LSD	1 %	0.020		
***LSD	0.1 %	0.030		

LSD - least significant difference

The fertilizing elements in different combinations achieve optimal insurance with Pt. (Table 5). Thus, the control variant in ternary combination (NPK) achieves 0.273% Pt (optimal for this phenophase), followed by the variants in binary complex NP (T2) with 0.259% Pt, PK (T3) with 0.258% Pt and the P variant (T5) with 0.254% Pt.

Table 5. The influence of the fertilizer combinations on the evolution of Pt (%) in leaves of Fetească albă variety

Fertilizer combinations		P_t (%)	% increase	Differences
Control	NPK	0.273	100.0	Mt
T1	NK	0.225	82.6	-0.047
T2	NP	0.259	94.8	-0.014
T3	PK	0.258	94.5	0.015
T4	N	0.226	82.9	-0.046
T5	P	0.254	93.1	-0.018
T6	K	0.225	82.6	-0.047
*LSD	5 %	0.105		
**LSD	1 %	0.139		
***LSD	0.1 %	0.181		

LSD - least significant difference

The research undertaken for the dosages of total potassium in the leaves (Table 6) highlighted that for dosage levels of fertilizers D1 and D2, the values recorded were below optimal, respectively values below 0.9% Kt. The D3 level was slightly above the optimum, respectively 0.909% Kt, being statistically significant.

Table 6. The influence of the fertilizer dose on the evolution of Kt (%) in the leaves of Fetească albă variety

Dose (kg s.a./ha)		Kt (%)	% increase	Differences
Control	0	0.876	100.0	Mt
D1	0.5	0.883	100.9	0.007
D2	1	0.886	101.2	0.010
D3	1.5	0.909	103.8	0.033
*LSD	5 %	0.028		
**LSD	1 %	0.040		
***LSD	0.1 %	0.060		

LSD - least significant difference

The method of combining the fertilizing elements (Table 7) brings the Kt content values around the optimal values for the NK (T1) and K (T6) variants with 0.900% Kt.

Table 7. The influence of the fertilizer combinations on the evolution of Kt (%) in leaves of Fetească albă variety

Type of fertilizer		Kt (%)	% increase	Differences
Control	NPK	0.914	100.0	Mt
T1	NK	0.900	98.4	-0.013
T2	NP	0.870	95.2	-0.043
T3	PK	0.899	98.3	-0.015
T4	N	0.870	95.1	-0.044
T5	P	0.868	94.9	-0.046
T6	K	0.900	98.4	-0.013
*LSD	5 %	0.017		
**LSD	1 %	0.023		
***LSD	0.1 %	0.029		

LSD - least significant difference

In Table 8, it can be noted the lower value of the total acidity in the must, in the case of administration of fertilizers in minimum doses compared to the use of optimal and maximum doses.

The administration of 0.5 doses of fertilizers, D1, contributes to obtaining a concentration of 5.27 g/L H_2SO_4, (distinctly significant), a value lower than that of the level of 1 dose, D2 (5.33 g/L H_2SO_4) and 1.5 doses, D3 (5.36 g/l H_2SO_4), both provided very significantly (Table 8).

Table 8. The influence of the fertilizer dose on the total titratable acidity (g/L H_2SO_4) in musts of Fetească albă

Dose level		Acid. (g/L)	% increase	Differences
Control	0	5.22	100.0	Mt
D1	0.5	5.27	101.0	0.054**
D2	1	5.33	102.0	0.109***
D3	1.5	5.36	102.7	0.144***
*LSD	5 %	0.036		
**LSD	1 %	0.052		
***LSD	0.1 %	0.077		

LSD - least significant difference

With the administration of fertilizers in combination NK (T1), the total acidity in the must rises to the value of 5.44 g/L H_2SO_4 (by 0.107 g/L H_2SO_4 more than the control version, fertilized with NPK), statistically very significantly ensured. At the opposite pole, the option of using phosphorus and potassium unilaterally, lowers the total acidity to the level of 5.23 and 5.24g/L H_2SO_4, respectively (Table 9).

Table 9. The influence of the fertilizer combinations on the total titratable acidity (g/l H_2SO_4) in musts of Fetească albă

Type of fertilizer		Acidity (g/L)	% increase	Differences
Control	NPK	5.33	100.0	Mt
T1	NK	5.44	102.0	0.107***
T2	NP	5.26	98.67	-0.071
T3	PK	5.25	98.54	-0.078
T4	N	5.32	99.83	-0.009
T5	P	5.23	98.20	-0.096
T6	K	5.24	98.41	-0.085
* LSD	5 %	0.040		
**LSD	1 %	0.053		
***LSD	0.1 %	0.069		

LSD - least significant difference

CONCLUSIONS

Foliar diagnosis, as a research method, plays an important and powerful role in the control of mineral impurities and in its adjustment, depending on the production directions. For the Fetească albă variety, located in a northern border area, fertilization with macroelements influences its state of insurance, in direct relation with the production quality, respectively with the acidity in the must.

Corollary to the research undertaken, it can be stated that along with the optimal doses of fertilizers, the minimum doses tend to ensure the life of the vine with macroelements, at sometimes optimal levels, statistically significant or not, as well as the acidity in the must, which induces the idea of their use, for protection of the nutrition environment.

Nitrogen, phosphorus and potassium influence the acidity in the must, with values that are specific to the variety. The NPK and NK fertilization relationship ensures acidity values, statistically confirmed and which reinforce in the studied area, the direction of use of this variety as being for quality wines with designation of origin and quality levels.

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GLOBAL RADIATION AND ITS IMPORTANCE FOR WHITE (YOUNG) WINE QUALITY IN THE JIDVEI WINE-GROWING CENTER

Liana-Maria ROPAN[1], Raluca-Iuliana TRUŞCĂ (POPESCU)[1, 2], Nicolae GIUGEA[1], Alina-Mariana ȘIMON[3], Claudia-Adriana HORȘIA[4]

[1]University of Craiova, Faculty of Horticulture, 13 Alexandru Ioan Cuza Str., Craiova, Romania
[2]Research and Development Station for Viticulture and Winemaking Drăgăşani, 64 Regele Ferdinand Str., Drăgăşani, Romania
[3]Agricultural Research-Development Turda, 27 Agriculturii Str., Cluj, Romania
[4]Jidvei Research Department, 45 Garii Str., Jidvei, Romania

Corresponding author email: raluk_trusk@yahoo.com

Abstract

Global warming and climate change are leading to disruptions in the viticultural ecosystem, with grapevine varieties having to modify their biological life cycle, in particular to adapt to a shorter growing season, with consequences for harvest quality. The study was carried out between 2019 and 2021 in the Jidvei wine-growing center, Târnave wine-growing region in Romania. The Fetească regală variety, the most cultivated white grape variety in Romania, was studied. The compounds in grape juice have a small amount of energy in their molecule, estimated using the heat of reaction. Their synthesis in a balanced relationship is with the climatic requirements of the biotope, the place where the vine grows and ripens, to which abiotic factors are added. In the first part it was necessary to evaluate the climatic resources. Also, the estimation of solar radiation was carried out with the help of the global radiation indicator and the coefficient of conversion of solar energy into chemical energy in life was calculated. In the second part, the grape variety Fetească regală was analyzed. The applied research methods were the ANOVA analysis and the Pearson method. Through the analysis of variance (ANOVA), the smallest significant differences - LSD - (5%, 1% and 0.1%) were established for the parameters of grapes at full maturity: sugars and total acidity. The ANOVA analysis was also applied for the following parameters of young wine: alcohol concentration, reducing sugar, total acidity in tartaric acid, non-reducing dry extract. Using linear regression and correlation coefficient, several trends were established between global radiation and analyzed variables. It was found that global radiation and grape parameters (sugars and total acidity) were very strongly associated. Stability relationships between global radiation and several young wine parameters (alcoholic concentration, total acidity and non-reduced dry extract) also revealed very strong relationships. The limited data for only three years (2019-2021), revealed insignificant statistical correlations, but the linear relationships between several variables suggest continuing the study for more years.

***Key words**: analytical characteristics, climate resources, ecosystem, energy flow, global radiation, variety, grapevine.*

INTRODUCTION

Sudden changes in temperature, frost, frost and excessive heat during the growing season can adversely affect production and quality.

Giugea (2001) appreciates that climatic factors are the first to respond and impose restrictions on the economic culture of the grapevine.

The vine is an active component of the viticultural ecosystem, and through its activity, as a functional component, it modifies the biotope and the microclimate (Malschi, 2014).

Cosmulescu (2015) appreciates that the vine receives essential mineral resources for growth and development from the outside. Through the exchange of substance and energy transfer, it finally has the capacity to produce, the capacity to care is expressed through its biological and productive potential.

Calo et al. (1997) carried out privileged determinations on the influence of climatic factors such as temperature, precipitation and solar radiation in triggering the vegetation and ripening phases of grapes. There is a correlation between weather conditions and sugar accumulation, shoot and leaf growth.

A decrease in global radiation can reduce the concentration in sugars or increase the total acidity in the same proportion. In order for the photosynthetic activity of the leaf apparatus to be intense, at least 70% of the leaves must be exposed to direct solar radiation. Severe water stress negatively influences photosynthesis by closing the stomata, reducing the accumulation of sugars and aromatic compounds (Irimia, 2012).

Higher values of solar radiation accelerate the process of budbreak, flowering and ripening of the grapes. The same values extend the vegetation period, have a better effect on the maturation of the wood and the winter hardiness of the vines (Oprea, 1995).

The flow of energy in a viticultural ecosystem is ensured by electromagnetic energy of solar radiation, in two forms: caloric radiation and radiation used for photosynthesis. As a result of this process, organic substances are produced and oxygen from the atmosphere is regenerated. Part of the radiation is reflected on the surface of the leaves, part passes through the leaves and another part is used in physiological processes (Malschi, 2014).

The studies carried out by Pap & Bozac, (1982), highlight the fact that of the total solar radiation, 43% is lost through reflection and diffusion, and 57% is absorbed and transformed into other forms of energy. Out of the amount of 57%, only 43% radiation reaches the soil and water surface in the form of direct and diffuse radiation.

Radiation from the visible spectrum (wave length 0.7-0.3μ), has an energetic and informational function for the viticultural biosystem. In the vine, the amount of solar energy is converted into a useful biological product (grapes) (Olteanu & Mărăcineanu, 2008).

The vine uses the caloric energy and the light energy of solar radiation. Differences greater than 15-20% of global radiation correlated with differences in slope and land exposure create differences for the development of phenophases, in increases in production and quality (Oşlobeanu et al., 1991).

In Romania, the distribution in the north is 107 $Kcal/cm^2$ (Oprea, 1995).

MATERIALS AND METHODS

The Târnavelor vineyard is located in the hydrographic basin of the two rivers, Târnava Mică and Târnava Mare, more precisely between parallels 45°57' and 46°32' north latitude and between meridian 23°52' and 24°48' east longitude. Also between these coordinates, north latitude 46°19' and east longitude 24°14', is the Jidvei wine-growing center.

The climatic resources of the Jidvei wine-growing region placed in value, place the Jidvei wine-growing center in the wine-climatic zone A_1 (Blaj-Jidvei), the area with specific climatic characteristics (Teodorescu et al., 1987).

The climate conditions in the Jidvei wine-growing center, during the study period (2019-2021), were established based on a complex of meteorological factors: temperature, light, humidity, global radiation.

Primary data were collected from weather stations Jidvei, Blaj and Târnăveni.

They were processed mathematically, as the annual average of the air temperature and the annual amount of precipitation (Tables 1 and 2). During the vegetation period (April-September), the sum of the effective hours of sunshine, the amount of precipitation, the active heat balance was calculated (Tables 3 and 4).

The insolation fraction is calculated by the formula (Pap & Bozac, 1982):

$$F = d/D$$

The correlation between cloudiness and relative insolation was obtained by the formula (Pap & Bozac, 1982):

$$N = (1 - F) * 10.$$

The length of the period of active vegetation in the vine, expressed in days, to calculate using the temperature level of +10°C, spring and autumn biological zero.

The oenological aptitude index was calculated according to the formula (Teodorescu, 1987):

$$IAOe = \sum t^{o}a\ (^{o}C) + \sum ir - (\sum P\ (mm) - 250)$$

The biological material used was the Fetească Regală variety, a representative variety for the vine culture in the Târnave area, with good characteristics for high-quality white wines and base wines for sparkling wines.

The analysis of variance (ANOVA), posthoc test Least Significant Differences (LSD) - (5%, 1% and 0.1%) was applied for grape parameters (sugars and acidity) at full grape maturity. The same approach was applied for the young wine parameters: alcohol concentration, reducing sugar, total acidity in tartaric acid and non-reducing dry extract.

Through correlation analysis, the degree of association between solar radiation and the

mean of the variables under study was determined.
Another factor used in the study was solar radiation. The estimate cannot be made using the global radiation indicator, measured in W/m^2, by reading the average from the vegetation period (April-September, 2019-2021). The paper used the formula for the standard unit of measure Kcal/h*m^2 expressed by: 1 kcal/(h*m^2) = 1.1629800200033 W/m^2
The aim of the work was to highlight the influence of climatic factors and solar radiation on the quality of the young white wine of the Fetească Regală variety.

Table 1. Average air temperature, during 2019-2021, in the Jidvei wine-growing center

Years of experience	January	February	March	April	May	June	July	August	September	October	November	December	April-September average	Annual average
2019	-1.3	1.9	7.4	11.9	15.0	21.6	20.0	21.5	16.4	10.3	9.2	0.9	17.7	11.2
2020	-2.5	3.2	6.6	10.1	14.6	19.5	20.2	21.3	17.3	11.6	3.6	3.9	17.2	10.8
2021	0.2	0.4	3.4	8.3	14.9	19.5	22.6	19.8	14.3	8.2	4.6	2.2	16.6	9.8
The average 1986-2021	-2.2	0.3	5.0	10.6	15.4	18.9	20.6	20.2	15.4	9.8	4.1	0.9	16.8	9.8

Table 2. Amount of precipitation, in the period 2019-2021, in the Jidvei wine-growing center

Years of experience	January	February	March	April	May	June	July	August	September	October	November	December	Amount in IV-IX	The annual amount
2019	34.0	31.6	11.6	74.4	132.4	47.8	103.4	95.0	16.2	46.4	31.4	41.6	469.2	665.8
2020	3.6	45.2	46.0	18.8	65.4	208.6	69.0	112.2	102.0	61.6	20.4	27.0	576.0	779.8
2021	28.4	18.8	65.0	82.0	112.0	76.4	82.4	99.6	88.8	15.6	34.0	69.2	541.2	772.2
The average 1986-2021	19.3	18.3	24.8	48.9	72.8	91.8	76.0	58.0	53.6	39.1	26.1	28.2	401.0	557.0

Table 3. The sum of the effective hours of sunshine, during the vegetation period (1IV-30 IX), 2019-2021 (Târnăveni weather station)

Years of experience	April	May	June	July	August	September	Effective sunshine during the growing season (hours)
2019	194.8	171.6	304.9	279.5	306.7	234.2	1,491.7
2020	288.3	209.7	196.1	260.2	304.1	237.3	1,495.7
2021	165.3	236.1	280.6	327.2	277.8	211.2	1,498.2
The average 1986-2021	184.3	220.9	240.1	257.8	253.7	179.6	1,336.4

Table 4. The sum of active temperature degrees during the vegetation period (April-September), 2019-2021, in the wine-growing center of Jidvei

Years of experience	April	May	June	July	August	September	October	The sum of active temperatures during the growing season
2019	136.4	449.5	648.1	619.4	667.3	355.7	0	2,876.4
2020	43.1	452.1	583.7	626.2	658.9	518.9	199.2	3,082.1
2021	39.6	460.6	584.9	701.0	615.0	318.0	0	2,719.1

RESULTS AND DISCUSSIONS

Baducă Câmpeanu et al. (2012) confirm that climate changes modify the life cycle of the grapevine, this cycle occurring earlier than normal. Mărăcineanu (2011), states that the length of the vegetation period is influenced by the climatic characteristics of the respective year.

The statements are similar to the continuation obtained in our study, the period of active vegetation calculated in the Jidvei viticultural center, had an average of 156 days in the interval 2019-2021. The average was below the value of 170-180 days, from the Blaj, Jidvei and Turda vineyards confirmed by other researchers Călugăr (2011), Corbean (2011), Ciobanu (2012), Hașegan (2014) and Baciu et al., (2020).

The data analysis, in the period 2019-2021, (Table 5) confirms that the climate resources achieved value the favorable climate conditions for the economic culture of the vine.

The rich thermal resources were more than enough for the requirements of the vine, throughout the annual vegetation cycle (Table 1). Average annual precipitation fully satisfied the high-water requirements of grapevines relative to soil and atmospheric moisture (Table 2).

The light was sufficient, throughout the vegetation period, useful in the sequence of growth and fruiting phenophases and for the entire vegetation period (Table 3).

The active heat balance registered during the period of active vegetation made it possible to go through normal biological and physiological processes from the life of the vine (Table 4).

In our study, the insolation fraction was used to measure the level of effective insolation. According to this correlation, during the vegetation period the maximum level was reached in August (Table 6).

The results in Table 7, show that depending on normal to the sum of the hours of effective sunshine, during the vegetation period, appreciates very good years, of quality, in the three years for the vine culture.

The data analyzed in the normal operation of the amount of precipitation during the vegetation period, estimates a favorable average for the vines (Table 8).

The oenological aptitude index (IAOe) reached an average value suitable for the growing conditions of white wine varieties. The achieved value falls within the optimal limits (3700-5200) for viticulture in Romania. The value of this index was lower than the data argued from the Jidvei and Turda area by other collaborators, Ioia (2009), Baciu et al. (2020).

Bucur & Dejeu (2022), state that short, medium and long-term adjustment strategies are needed for viticulture practice, considering climate change and local biotope conditions.

Table 5. Climatic resources achieved in the period 2019-2021, in the Jidvei wine growing center

Studied years 2019-2021	The amount of precipitation yearly (mm)	Medium temperature yearly (°C)	Active thermal balance (ºC)	Actual duration of the brightness of the sun April-September (hours)	Precipitation April-September (mm)	Oenological aptitude index
2019	665.8	11.2	2,876.4	1,491.7	469.2	4,148.9
2020	779.8	10.8	3,082.1	1,495.7	576.0	4,251.8
2021	772.2	9.8	2.719.1	1,498.2	541.2	3,926.1

Table 6. Insolation fraction during the vegetation period (April-September), 2019-2021, in the wine-growing center of Jidvei

Studied years	April	May	June	July	August	September	Insolation fraction
2019	0.48	0.37	0.65	0.59	0.70	0.62	0.57
2020	0.71	0.45	0.42	0.55	0.70	0.63	0.57
2021	0.41	0.51	0.59	0.69	0.63	0.56	0.57

Table 7. Climatic favorability for studied years (2019-2021) in relation to the reference period (1986-2021) for the amount of sunshine during the vegetation period (April-September), in the Jidvei wine-growing center

Studied years	The amount of actual sunshine, (hours)	Average 1986-2021, (hours)	Deviation from normal (%)
	V	N	V/N*100-100
2019	1,491.7	1,336.4	+11.6
2020	1,495.7	1,336.4	+11.9
2021	1,498.2	1,336.4	+12.1

Table 8. Climatic favorability for studied years (2019-2021) in relation to the reference period (1986-2021) for rainfall during the growing season (April-September), in the Jidvei wine-growing center

Studied years	The amount of precipitation (mm)	Average 1986-2021 (mm)	Deviation from normal (%)
	V	N	V/N*100-100
2019	469.2	401.0	+17.0
2020	576.0	401.0	+43.6
2021	541.2	401.0	+35.0

Table 9. Cloudiness during the growing season, 2019-2021, in the Jidvei wine-growing center

Studied years	April	May	June	July	August	September	Cloud
2019	5.21	6.31	3.54	4.14	3.00	3.78	4.34
2020	2.94	5.50	5.85	4.54	3.04	3.68	4.32
2021	5.95	4.93	4.06	3.13	3.65	4.38	4.31

The amount of radiant solar energy received by the earth's surface during a year depends on latitude, adverse weather factors, seasons and relief. Water vapor and carbon dioxide are decisive factors for the accumulation of caloric energy through the absorption of infrared radiation. Climatically, cloudiness influences solar and terrestrial radiation (Pap & Bozac, 1982).

From the results of the study, the values obtained for the global radiation indicator between April and September, in the 3 years of the study, confirm the data in Table 10.
The achieved average was 1195 Kcal/h*m^2.
Similarly, these achieved values are closely related to the cloudiness during the vegetation period (Table 9).

Table 10. Estimation of the influence of solar radiation using the global radiation indicator, 2019-2021, in the wine-growing center of Jidvei

Studied years	Global radiation (W/m^2)							April-September (Kcal/h*m^2)
	April	May	June	July	August	September	April-September	
2019	202.0	205.2	292.9	266.4	253.7	189.0	1,409.2	1,212
2020	240.2	217.3	239.0	255.1	252.6	190.6	1,394.8	1,200
2021	179.0	233.1	276.0	278.3	229.1	168.8	1,364.3	1,173
Average 2019-2021	207.1	218.5	269.3	266.6	245.1	182.8	1389.4	**1,195**

As Malschi (2014) says, it was taken into account that the vine stores the amount of solar energy, to transform it into organic matter through the process of photosynthesis.
(Seybold, 1932), shows that the radiation with wavelengths between ƛ= 0.7-0.3ų involved in photosynthesis activity is estimated at 50% of the total global radiation received from the sun.
Georgescu (1991) confirms that the leaf is the place where solar energy is converted into chemical energy.
The active leaf apparatus retains 80% of the radiation included in the chlorophyll assimilation processes and another 10% of the inactive radiation, Oșlobeanu et al. (1991).

The results of the indirect calorimetry studies in the Banu Mărăcine wine center (Dealurile Craiovei vineyard) demonstrated a conversion coefficient of solar energy into chemical energy of 0.84%. The same study reveals the cultivation of varieties with a high potential for converting this caloric energy into organic matter to capitalize on the energetic conditions in the given viticultural ecosystem. (Olteanu, 1991).
Our research demonstrated that in the Jidvei wine-growing center, a conversion coefficient of solar energy into chemical energy of 0.58% was obtained to the vine (Table 11).

We believe that it was not necessary to calculate for each year of the study, in part, this conversion coefficient, because close values of the global radiation resulted in the three years analyzed.
By comparison, this coefficient is lower than the value of the conversion coefficient in the Banu Mărăcine wine area. Oșlobeanu et al. (1991), state that the amount of global radiation received from the sun is smaller in the vineyards of Târnave and recommends the cultivation of cultivars with certain morphological characteristics of the leaf or internodes that improve the penetration of solar energy for the most leaves on a shoot.

Table 11. Conversion of solar energy into chemical energy in the vine, 2019-2021, in the Jidvei viticultural center ($Kcal/h*m^2$)

Steps for converting solar energy into chemical energy	Author	Scoring and calculation method	$Kcal/h*m^2$
(A) Global radiation (direct + diffuse), in the interval (April - September), 2019-2021, value calculated according to the data obtained in Table 10	-	A (average)	1195
(B) The radiation contained in the process of photosynthesis estimated at 50% of the total radiation (from A)	Oșlobeanu and others (1991)	B = (A x 50)/100	598
(C) Radiation intercepted by leaf apparatus estimated 50% of that used in photosynthesis (from B)	Oșlobeanu and others (1991)	C = (B x 50)/100	299
(D) Incident radiation retained by active leaf apparatus 80% (from C)	Oșlobeanu and others (1991)	D = (C x 80)/100	239
(E) Radiation not participating in photosynthesis, 10% of that intercepted by the leaf apparatus (from D)	Oșlobeanu and others (1991)	E = (D x 10)/100	24
(F) Total absorbed active radiation	Oșlobeanu and others (1991)	F = D - E	215
(G) Energy value of grape production for 10 t/ha	Olteanu & Mărăcineanu, 2008	G = C / D	1.25
The coefficient of conversion of solar energy into chemical energy, % (H)	Oșlobeanu and others (1991)	H = (G / F) x 100	**0.58**

Table 12. Influence of climatic conditions on sugars (g/L) and total acidity (g/L H_2SO_4) at full maturity of grapes, in the wine-growing center of Jidvei

Experimental variants	**Analyzed parameters**	**Difference**	**Statistical significance**
	Sugars (g/L)		
Average	213.00	0.00	Mt
2019	214.00	1.33	--
2020	215.67	2.67	--
2021	209.00	-4.00	--
DL (p 5%)		7.70	
DL (p 1%)		10.44	
DL (p 0.1%)		14.08	
	Acidity expressed as H_2SO_4 (g/L)		
Average	5.98	0.00	Mt
2019	5.70	-0.29	--
2020	5.69	-0.29	--
2021	6.56	0.58	--
DL (p 5%)		0.61	
DL (p 1%)		0.84	
DL (p 0.1%)		1.18	

The studies carried out by Oprea (1995), reveal the fact that during the ripening of grapes, sugars accumulate progressively, until they reach a maximum level at full maturity. Exceptions are years with excessive pedological drought when the sugar accumulation process becomes very slow, and years with excess moisture in which the sugar concentration is reduced by dilution. The glucoacidimetric index, as a ratio between sugar and acidity, is decisive for wine grapes.

(Georgescu, 1991), estimates that of the total sugars, 90% are stored in grapes and 10% in the other annual organs.

The action of the climatic conditions in the three experimental years, in the Fetească Regală variety, for sugars and total acidity at full maturity of the grapes, is presented in Table 12. From the analysis of the obtained values, in the year 2021, it emerges that the sugars accumulated at full maturity registered a decrease of -4 g/l below the average of the 3 years taken as a control. The concentration in sugars was reduced by dilution, the year 2021 being considered normal from a thermal point of view and very rainy.

Compared to the years 2019 and 2020, the accumulated sugar values are higher than the 3-year average, without statistical assurance.

Regarding the acidity in the must, from the data presented in Table 12, insignificant differences emerge from a statistical point of view compared to the control. The biggest big difference +0.58 g/l H_2SO_4, compared to the average was registered in 2021.

The variability of several parameters of young wine (alcohol concentration, reducing sugar, acidity in tartaric acid, non-reducing dry extract), in the period 2019-2021, is presented in Table 13.

Table 13. The influence of climatic conditions on the analytical characteristics of young wine, in the Jidvei wine-growing center

Experimental variants	**Analyzed parameters**	**Difference**	**Statistical significance**
	Alcohol concentration (% vol.)		
Average	12.26	0.00	Mt
2019	12.47	0.21	**
2020	12.29	0.03	--
2021	12.02	-0.24	00
DL (p 5%)		0.12	
DL (p 1%)		0.17	
DL (p 0.1%)		0.24	
	Reducing sugar g/L		
Average	4.31	0.00	Mt
2019	6.04	1.73	**
2020	3.12	- 1.19	--
2021	3.78	- 0.53	--
DL (p 5%)		1.21	
DL (p 1%)		1.64	
DL (p 0.1%)		2.21	
	Total acidity expressed as tartaric acid g/L		
Average	6.98	0.00	Mt
2019	6.69	-0.29	--
2020	6.84	-0.14	--
2021	7.41	0.43	**
DL (p 5%)		0.29	
DL (p 1%)		0.41	
DL (p 0.1%)		0.57	
	Non-reducing dry extract g/L		
Average	17.42	0.00	Mt
2019	17.74	0.32	--
2020	17.32	- 0.11	--
2021	17.22	- 0.21	--
DL (p 5%)		0.32	
DL (p 1%)		0.44	
DL (p 0.1%)		0.61	

The same author shows that, the climatic favorability according to the normal rainfall, divides the harvest years into favorable, when less than 9.7% compared to the average is recorded, and unfavorable when the values are above 10.7%.

In our study, in the three years, determinations were made of the influence of the climatic favorability of the harvest years on the studied parameters.

In 2019, the alcohol concentration registered increases of +0.21 vol % compared to the average of the years taken as a reference, a distinctly positive statistically significant difference. At the opposite pole was the year 2021, a year in which a distinctly negative difference was registered, statistically assured (-0.24 vol %).

Similar results were also obtained for reducing sugar, in 2019 the difference obtained compared to the average of the reference years was +1.73 g/l, being statistical as significantly positive.

Regarding the total acidity in tartaric acid in 2021, this parameter increased by +0.43 g/l, compared to the average of the 3 years taken as a control, a difference ensured statistically as significantly positive.

In the case of the non-reducing dry extract, in 2019, the difference obtained compared to the medium of the control years was not statistically significant.

Correlation analysis was used to identify the strength of the relationship between the studied variables and establish their statistical significance.

At the full maturity of the grapes, the following results were highlighted:

From the analysis of the data (Figure 1), it follows that there is a very high positive relationship between solar radiation and sugar content, but statistically insignificant, given by a correlation coefficient (r = 0.8794).

The analyzed data on the correlation between solar radiation and averages of total acidity (Figure 2) show that there is an inverse very strong relationship between these two variables (r = -0.951), but statistically insignificant.

The following results were evident for the young wine:

From the analysis of the data presented in Figure 3, it appears that there is a very strong association between solar radiation and alcohol concentration, but statistically insignificant, given by a correlation coefficient (r = 0.9946).

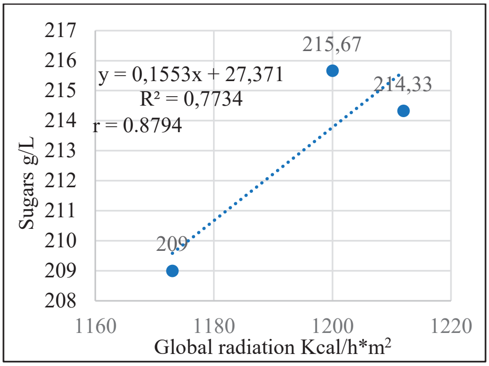

Figure 1. Correlation between solar radiation and sugar content at full maturity of grapes, in the Jidvei wine-growing center

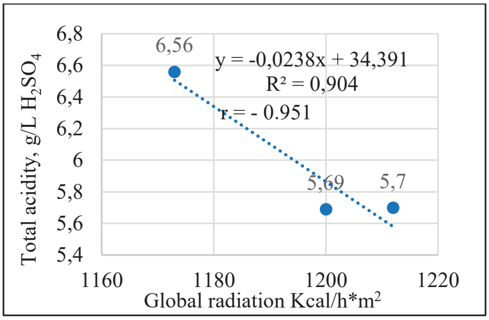

Figure 2. Correlation between solar radiation and total acidity at full ripeness of grapes, in Jidvei viticultural center

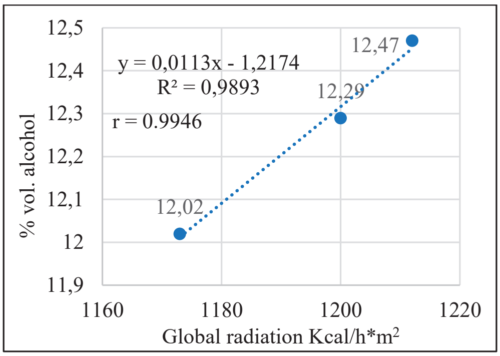

Figure 3. Correlation between solar radiation and alcohol concentration in young wine

Between solar radiation and reducing sugar, Figure 4, there is a moderate positive relationship, the correlation coefficient with the value of (r = 0.5741).

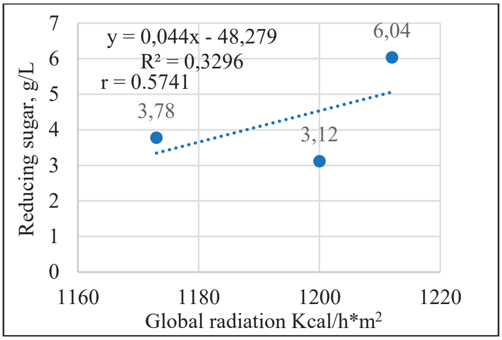

Figure 4. Correlation between solar radiation and reducing sugar in young wine

There is a very strong inverse relationship between solar radiation and total acidity in tartaric acid (Figure 5), statistically insignificant (r = -0.994).

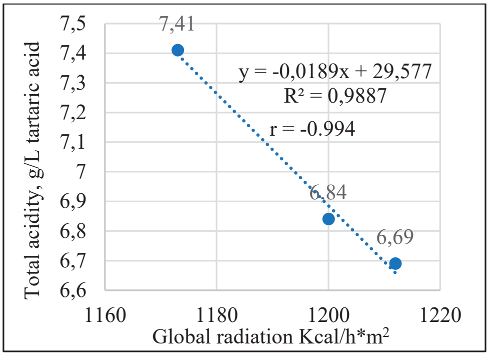

Figure 5. Correlation between solar radiation and total acidity (tartaric acid g/l) in young wine

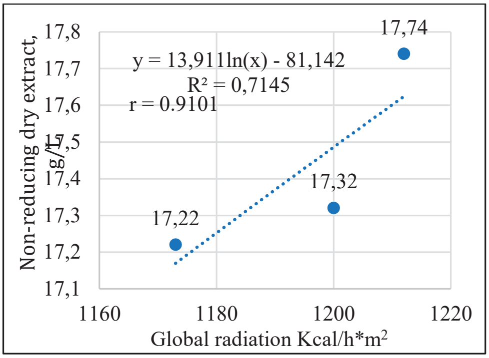

Figure 6. Correlation analysis between solar radiation and non-reducing dry extract (g/l) in young wine

Relationship between solar radiation and non-reducing dry extract (Figure 6), revealed a very strong positive relationship (r = 0.9101), but statistically insignificant.

CONCLUSIONS

From the synthesis of the work during 2019 and 2021, it follows that in the Jidvei wine-growing center the assessed climatic resources are very favorable for the cultivation of varieties for white wines and the oenological aptitude index reached an average value suitable for the growing conditions of white wine grape varieties.

The applicative potential was argued by the climatic favorability of the Jidvei wine-growing area, evaluated as favorable for the economic culture of the vine.

The lower value of the chemical energy conversion coefficient obtained in our study emphasizes the value of this index in everyday practice in viticulture. As a recommendation, sun cultivation with characteristics that allow the leaf to capture more solar energy on the one hand and at the same time to capitalize on the maximum ecoclimatic conditions in the Târnavel area, with positive results in the phenophases of growth and fruiting in the vine.

In the Fetească Regală variety, the climatic conditions during the three years of the study did not significantly influence the content in sugars and acidity at the time of harvesting the grapes at full maturity.

Alcohol content and total acidity of young wine are the main parameters influenced by climatic conditions.

The reduction of sugars in wines was not influenced by climatic conditions, the differences resulting in the three years of the study were statistically insignificant. Fermentation conditions (different yeast strains, temperature, nutrients, exposure to oxygen during fermentation) are the uncontrolled factors responsible for different final concentrations, as they were not completely turn into alcohol. The young wine was fermented almost to dryness.

From the research data, it was found that global radiation and grape parameters were very strongly associated. Between global radiation and several young wine parameters also revealed very strong relationships. In all graphs presented, Pearson „r" takes values below the critical value from the statistical tables, which means that all these correlations are statistically insignificant, even if the strength of the association suggests a relationship between the studied variables.

Limited data for only three years (2019-2021) revealed insignificant statistical correlations, but the linear relationships between several variables suggest continuing the study for more years.

ACKNOWLEDGEMENTS

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***Analysis and Control Laboratory, Jidvei Research Department, RO

***Jidvei SRL network of private weather stations, Jidvei weather station, RO

***Regional Meteorological Center Transylvania South Sibiu, weather station Blaj, RO.

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EVALUATION OF OENOLOGICAL POTENTIAL OF CLONAL SELECTIONS OF OBTAINED AT THE STEFANESTI WINE CENTER

Dorin SUMEDREA[1], Alina FLOREA[1], Andrei TANASE[1], Anca ONACHE[1], Adrian ASĂNICĂ[2]

[1]National Research & Development Institute for Biotechnology in Horticulture Stefanesti Bucharest-Pitesti Street, no. 37, Stefanesti-Arges, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: alinaflorea964@gmail.com

Abstract

The aim of this study is to characterize and differentiate the main physico-chemical parameters, the evolution of the anthocyanin profile and the total polyphenol content of white and red wines obtained from clones of the varieties 'Feteasca Regala', 'Muscat Ottonel', 'Sauvignon Blanc', 'Burgund', 'Cabernet Sauvignon' and 'Merlot'. For the production of white wines, the clones 'Fetească Regală 72 St', 'Muscat Ottonel 16 St', 'Sauvignon Blanc111 St' and 3 other clones for the production of red wines, 'Burgund 86 St', 'Merlot 202 St' and 'Cabernet Sauvignon 131 St' were studied. The results showed that the red wines produced from the 'Burgund 86 St', Merlot 202 St' and 'Cabernet Sauvignon 131 St' clones outperformed the standard wines in terms of total anthocyanin and polyphenol content. In all the studied clones, the physico-chemical indicators, the alcohol concentration and the tannins in the wines, recorded higher values than the control varieties.

***Key words**: Vitis vinifera L., climate variability, clone, physico-chemical parameters.*

INTRODUCTION

Globally, the trends in viticulture are towards new vine varieties with genetic resistance and clonal selections of widespread varieties that are better adapted to climate change and that give quality grapes and better yields. The genetic diversity of the *Vitis* genus is a valuable resource for adapting to future climate change, but for the production of high-quality wines, both complex selection and aging programs are needed, as well as winemaking experiments with the resulting biological material (Faria, 2020). Conditions of increased water stress decrease grape production and winemaking yields, aspects that are further reflected in the economic part of the wine sector. At the same time, global warming increases the probability of extreme, unwanted climatic events (hail, floods, low temperatures during the flowering period, late spring frost, etc.). Under these conditions, appropriate vine adaptation strategies are needed to be able to continue producing high quality wines and to preserve varietal and terroir typicity in a changing climate. The quality of biological material remains a valuable resource for the implementation of these strategies (Cornelis van Leeuwen and Darriet, 2016). For a centralized record of grapevine varieties in Europe, in 1984, it was proposed to establish the *International Catalog of Vitis Varieties (VIVC)*, with the Institute for Grapevine Breeding Geilweilerhof, from Germany, as coordinator. The grapevine genetic resources database concept was supported by the International Board for Plant Genetic Resources (IBPGR) and the OIV. Later, other databases for this genre were created in Bulgaria, Czech Republic, France, Italy, Slovenia and Spain. Today, at the European level, the database presents information about approximately 23,000 varieties, hybrids and species of the genus *Vitis vinifera* existing in grapevine collections and/or described in specialized works. It is a useful source of information for the management of germplasm collections, as well as for vine growers and producers of wine and wine-derived products (Popescu et al., 2018). Through the clonal selection operations carried out over time, in Romania 54 clonal selections were obtained and promoted in practice, 9 from table varieties and 45 clonal selections from wine varieties. Among the clonal selections of winemaking varieties, about 35 clonal selections are from white wine varieties and 10 clonal selections are from red wine varieties (Marin et al., 2018). At NRDIBH Ștefanești, in the last decades, an intense activity of improving the vine was carried out, which led to the achievement of notable results. Following the long-term activity, at NRDIBH Ștefanești, new clones were obtained of most of the old varieties from the basic assortment, as well as new varieties in different production directions.

The present study focused on the analysis of three clones for white wines ('Muscat Ottonel 16 St', 'Fetească Regală 72 St', 'Sauvignon Blanc 111 St'), and 3 other clones intended for obtaining red wines ('Burgund 86 St', 'Merlot 202 St' and 'Cabernet Sauvignon 131 St') which are more spreaded in the Ștefãnesti-Argeș wine-growing area. This study revealed information about the quality of white and red wines obtained from clones created at NRDIBH Stefanești, Romania, useful for their further promotion.

MATERIALS AND METHODS

The experiment was located at NRDIBH Stefanești. During the research, 3 clones from white wine cultivars ('Fetească Regală 72 St', 'Muscat Ottonel 16 St' and 'Sauvignon Blanc 111 St') and 3 clones of three red wine cultivars ('Burgund 86 St', 'Merlot 202 St' and 'Cabernet Sauvignon 131 St') were studied. The varieties were grafted on the rootstock Oppenheim 4 (SO-4), the leading form being Guyot on the stem. Twelve plants from each clone were randomly marked in four rows. The analyzed white and red wines were produced under the same microvinification conditions at INCDBH Ștefanesti, Arges County. After the second infusion, the wines obtained from the studied clones, the 2020 and 2021 production respectively, were filtered and bottled. The determination of the physico-chemical parameters of the wines established from the studied clones was carried out in the Oenology laboratory with the help of Automatic oenological analyzer MINDRAY BS-200 connected to the computer, hydrostatic balance GIBERTINI, spectrophotometer UV/Visible SPECORD 205, electronic pH-meter, GIBERTINI digital still, Salleron ebulometer.

The spectrophotometric analyses of the red and white wines focused on the content of total anthocyanins and total polyphenols. The content in total polyphenols was determined by the Folin-Ciocâlteu method, and the expression was done in relation to a calibration curve with gallic acid, the results being expressed in mg (GAE) total polyphenols/l sample. The determination of the total anthocyanin content, expressed in mg/l, was carried out by the method of Ribereau Gayon-Stonestreet, 1976).

The region is characterized by a humid temperate-continental climate, with a mean annual temperature (Tmean) of 9.6°C and an amount of precipitation of 671.8 mm for the period 1979-2019 not evenly distributed throughout the year. The weather data were collected from the meteorological platform NRDBH Stefănești, geographical coordinates 44°51'N and 24°57'E. Reference varieties were compared with their clonal selections using the individual t-test, for a statistical significance level of 5%. All data were entered into an excel spreadsheet and then imported, processed and interpreted using the statistical program SPSS.14.0.

RESULTS AND DISCUSSIONS

Air temperature (lower thresholds, maximum thresholds, optimum level, critical moments) has a dominant influence on the vine according to the vine phenology dynamic. We had available for this analysis daily climatological databases for the experimental period (2020-2021), compared to the multi-year values (1979-2019). Average monthly temperatures were used to calculate a set of bioclimatic indices commonly used in viticulture. Table 1 describes the evolution of climate indicators from the experimentation period (2020-2021) compared to the multiannual average (1979-2019), at NRDIBH Ștefanesti. In the study period (2020-2021) higher temperatures were recorded compared to the multi-year average, especially in terms of the maximum and minimum annual temperatures (Table 1), and during the winter period much higher temperatures from one year to another (Table

1). Compared to the long-term averages (1979-2019), the average annual air temperature was higher by 1.25°C in 2020, respectively by 0.82°C in 2022. From table 1, it can be seen that 2020 was an extremely dry year, the precipitation during the vegetation period being far below the multi-annual average. It has been noted that in temperate regions that generally do not suffer from drought, a certain controlled deficit of water, during the ripening period, favors the organoleptic qualities of wine (Riou, 1994). However, in the study area, in the two analyzed years, the rainfall deficit in the vegetation period was 194.26 mm in 2020 and, respectively, 90.66 mm in 2022, compared to the multi-year average of 530.26 mm (2070-2019), influencing the main indicators of wine quality.

Table 1. Climatic indicators of the experimentation period (2020-2021) compared to the multiannual average (1979-2019), INCDBH Stefanesti - Arges

Climatic indicator	Multianual average (1979-2019)	2020	2021
Average annual temperature, °C	10.98	12.23	11.8
Average temperature in the growing season, °C (IV-X)	16.66	19.93	17.41
Average temperature in summer (ºC) (VI-VIII)	21.45	22.35	23.08
Average annual minimum temperature (ºC)	5.95	7.8	9.86
Absolute minimum temperature (ºC)	-18.72	-10.9	-13.9
Average January minimum temperature (ºC)	-7.58	-10.1	-10.9
Average annual maximum temperature (ºC)	21.29	23.72	23.84
Average July maximum temperature (ºC)	32.18	37.7	37.9
Annual total precipitation, mm	783.1	380	770.4
Total precipitation in the growing season, mm (IV-X), respectively for the years 2020-2021	531.26	337	440.6
The total precipitation in summer (VI-IX)	307.74	231.2	213.2

Evaluation of the physico-chemical indicators of the white wines

In both years of the study, the wines obtained from the 'Fetească Regală 72 St' clonal selection presented a higher alcohol content of 13.73 % vol. (2020), respectively 12.83% vol. (2021), compared to the reference cultivar which registered an alcoholic content of 12.20 % vol. (Table 2).

Alcohol content of the wines analyzed are much higher in 2020, compared to those obtained in 2021. This can be explained by the high temperatures that occurred in the climatic year 2020, and low precipitation until the end of autumn in the reference area with a deficit of 194.26 mm recorded (Table 1), allowing the accumulation of greater amounts of sugars in the grapes. Also, the clonal selection "Fetească Regală 72 St' recorded higher values of the non-reducing extract (18.42 g/l and 19.8 g/l respectively), compared to the control that reached a level of the non-reducing dry extract of 18.12 g/l (2020), respectively 18.32 (2021), the differences between them being statistically significant, in both years of study (Table 2). It is known that high values of non-reducing extract provide wines with consistency and personality (Hodor, 2011).

In the two analyzed years, the total acidity of the analyzed wine samples (Table 2) recorded values between 5.34 and 5.43 g/l tartaric acid in the clonal selection 'Fetească Regală 72 St' and 5.45-5.87 g/l tartaric acid in the reference cultivar the differences between them being significant. The clonal selection 'Muscat Ottonel 16 St' recorded a higher alcohol content and non-reducing dry extract than the analyzed control, the differences between them being significant (Table 3). The results obtained in 2020 regarding the acidity, respectively values of 4.85 highlighted in clone 'Muscat Ottonel 16 St' and over 4.59 g/l tartaric acid in the reference cultivar were similar to those presented by Chircu, 2014. Also, the results can be compared to those reported by de Bora et al. (2016) who reported a higher alcohol content, 'Fetească Regală' (13.80% vol.) and lower for 'Muscat Ottonel' (11.00% vol.).–The consistency of the wines given by the non-reducing dry extract and the concentration of the alcoholic (% vol) showed superior values for the clonal selection 'Sauvignon Blanc 111

St', compared to the reference cultivar, in both evaluation years (Table 4).
The volatile acidity of the wines obtained from the 'Fetească Regală', 'Muscat Ottonel' and 'Sauvignon Blanc' cvs. had values located within the accepted limits, but with higher values for their clones.

Table 2. Physico-chemical characteristics of the wines obtained from 'Fetească Regală 72St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Zah. Nereduc., g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	pH
2020						
'Fetească Regală' control reference	12.20±0.20^{b*}	18.12±0.08^{b}	1.8±0.20^{b*}	5.87±0.03^{b}	0.27±0.02^{b}	3.28±0.02^{b}
'Fetească Regală 72 St'	13.73±0.33^{a}	18.42±0.08^{a}	2.9±0.33^{a}	5.34±0.08^{a}	0.23±0.02^{a}	3.25±0.01^{a}
2021						
'Fetească Regală' reference	12,20±0.08^{a}	18.32±0.8^{b}	1.3±0.22^{b*}	5.45±0.08^{b*}	0.42 ±0.42^{b*}	3.28±0.02^{b}
'Fetească Regală 72 St'	12,83±0.08^{a}	19.8±0.12^{a}	2.7±0.28^{a}	5.43±0.38^{a}	0.21±0.033 ^{a}	3.25±0.01^{a}

*T-test for independent samples ($p<0.05$)

Table 3. Physico-chemical characteristics of the wines obtained from 'Muscat Ottonel 16 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Non-reducing sugar g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	pH
2020						
'Muscat Ottonel' reference	12.07±0.40^{b}	17.77±0.25^{b}	1.47±0.40^{b}	4.59±0.17^{b}	0.50±0.08^{b}	3.51±0.01^{a}
' Muscat Ottonel 16 St'	12.87±0.12^{a}	18.27±0.15^{a}	1.17±0.12^{a}	4.85±0.36^{a}	0.46±0.01^{a}	3.52±0.02^{a}
2021						
'Muscat Ottonel' standard	11.92±0.40^{b}	18.07±0.25^{b}	1.9±0.40^{b}	4.39±0.12^{b}	0.34±0.03^{b}	3.57±0.04^{a}
'Muscat Ottonel 16 St'	12.7±0.12^{a}	18.27±0.15^{a}	1.3±0.12^{a}	4.85±0.06^{a}	0.27±0.01^{a}	3.65±0.02^{a}

**T-test for independent samples ($p<0.05$)*

Table 4. Physico-chemical characteristics of the wines obtained from 'Sauvignon Blanc 111 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivar / clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Non-reducing sugar g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	pH
2020						
'Sauvignon Blanc' reference	13.97±0.55^{b*}	19.43±0.26^{b}	4.20±0.55^{b*}	4.90±0.07^{b}	0.47±0.02^{b}	3.46±0.01^{a}
'Sauvignon Blanc 111 St'	13.23±0.15^{a}	19.54±0.15^{a}	1.23±0.15^{a}	5.08±0.04^{a}	0.36±0.04^{a}	3.47±0.01^{a}
2021						
'Sauvignon Blanc' reference	13.8±0.55^{b*}	19.0±0.16^{b*}	5.3±0.55^{b*}	6.08±0.07^{a}	0.37±0.08^{b}	3.46±0.12^{b*}
'Sauvignon Blanc 111 St'	13.0±0.15^{a}	19.8±0.08^{a}	1.5±0.15^{a}	6.31±0.04^{a}	0.23±0.02^{a}	3.65±0.04^{a}

**T-test for independent samples ($p<0.05$*

Evaluation of physical-chemical indicators in red wines

All the clones for red wines exceeded the control variety in terms of the alcohol concentration in the wines, the non-reducing dry extract, as well as the total acidity in the wines.

From Table 5 it can be seen that the year of the study influenced the alcoholic concentration of the wines, total sugar and acidity.

The total acidity proved to be higher in the clonal selection 'Merlot 202 St' (5.13 AT g/l in 2020, respectively 6.0 AT g/l in 2021), than in the case of the standard cultivar (5.07 TA g/l, respectively 4.04 in 2021) (Table 6).

High temperatures accelerate decrease of grape acidity, mainly due to a faster degradation of malic acid (Buttrose et al., 1971).

The increase in grape sugar concentration and alcohol concentration is frequently reported in the literature (Bucur et al., 2019; Aloston et al., 2011; Duchêne and Schneider, 2016; Neethling et al., 2012).

The results obtained at the clonal section 'Cabernet Sauvignon 131 St' in 2020, of the total acidity in the wines (3.73) were also reported by Filip (2018) in a study on the chemical composition of three 'Cabernet Sauvignon' clones studied in the area of the Valea Calugareasca culture (Filip, 2018). These values obtained at NRDIBH Stefanesti for the 3 clones of red wines studied, compared with those obtained in other cultivation areas in the country, fall within the normal limits.

Table 5. Physico-chemical characteristics of the wines obtained from 'Burgund 86 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Non-reducing sugar g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	pH
2020						
Burgund reference	12.83±0.25^{b*}	28.93±0.06^{b}	1.93±0.25^{b*}	5.89±0.035^{a}	0.27±0.006^{b}	3.29±0.01^{b}
Burgund 86 St	13.08±0.08^{a}	29.20±0.10^{a}	3.98±0.08^{a}	6.44±0.07^{a}	0.25±0.007^{a}	3.26±0.006^{a}
2021						
Burgund reference	12.67±0.25^{b*}	22.9±0.06^{b}	1. 3±0.25^{b*}	5.93±0.035^{a}	0.48±0.006^{b}	3.42±0.01^{b}
Burgund 86 St	12.8±0.08^{a}	26.85±0.10^{a}	3,85±0.08^{a}	5.86±0.07^{a}	0.24±0.007^{a}	3.3±0.006^{a}

*T-test for independent samples ($p<0.05$).

Tabelul 6. Physico-chemical characteristics of the wines obtained from 'Merlot 2020 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Non-reducing sugar g/l.	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	pH
2020						
Merlot reference	10.80±0.28^{b*}	18.36±0.15^{a}	3.50±0.10^{b*}	5.07±0.038^{a}	0.71±0.04^{b}	3.81±0.015^{a}
Merlot 202 St	11.80±0.11^{a}	18.30±0.17^{a}	2.80±0.02^{a}	5.13±0.07^{a}	0.48±0.03^{a}	3.82±0.012^{a}
2021						
Merlot	10.80±0.10^{b*}	18.36±0.11^{a}	3.35±0.18^{b*}	4.07±0.038^{a}	0.71±0.09^{a}	3.59±0.015^{a}
Merlot 202 St	11.80±0.08^{a}	18.30±0.17^{a}	2.55±0.11^{a}	6.00±0.066^{a}	0.48±0.01^{b}	3.5±0.012^{a}

*T-test for independent samples ($p<0.05$).

Table 7. Physico-chemical characteristics of the wines obtained from 'Cabernet Sauvignon 131 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Non-reducing sugar, g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	pH
2020						
C. Sauvignon reference	12.77±0.26^{a}	18.47±0.21^{b}	2.2±0.04^{b*}	3.69±0.02^{a}	0.82±0.033^{b}	3.25±0.017^{b}
C. Sauvignon 131 St	12.80±0.1^{a}	21.82±0.11^{a}	2.7±0.1^{a}	3.73±0.025^{a}	0.69±0.021^{a}	3.43±0.08^{a}
2021						
C. Sauvignon reference	12.27±0.15^{b*}	22.6±0.30^{b*}	3.0±0.2^{a}	6.71±0.02^{a}	0.82±0.021^{a}	3.26±0.021^{b}
C. Sauvignon 131 St	12.50±0.08^{a}	26.02±0.11^{a}	3.2±0.1^{a}	7.19±0.025^{b}	0.71±0.021^{b}	3.44±0.01^{a}

*T-test for independent samples ($p<0.05$).

The content in total polyphenols in the analyzed white wines

Increasing temperatures negatively influence the accumulation of anthocyanins in grains (Montealegre et al., 2006), while strong solar radiation can have opposite effects. Also, higher concentrations of substances characteristic of muscat-type varieties (from the terpene family) were highlighted in naturally shaded grapes compared to grapes exposed to light radiation (Bertelli et al., 2002; Mattivi et al., 2006).

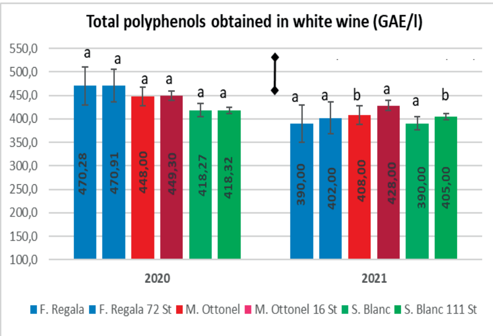

Figure 1. Content of white wines in total polyphenols from 'Fetească Regală', 'Mulscat Ottonel' and 'Sauvignon Blanc' clone selections compared to standard cultivars

Both the amounts of total polyphenols and the antioxidant activity recorded higher values in the wines obtained from the clonal selections studied, compared to the wines obtained from the control varieties, being higher in the 2020 crop year (Figures 1 and 3). The highest concentrations of total polyphenols were evident in the clone 'Fetească Regală 72 St' (470.91), in 2020, and the lowest were evidently in the 'Sauvignon Blanc' cultivar (390.0 GAE/l) in 2021 (Figure 1).

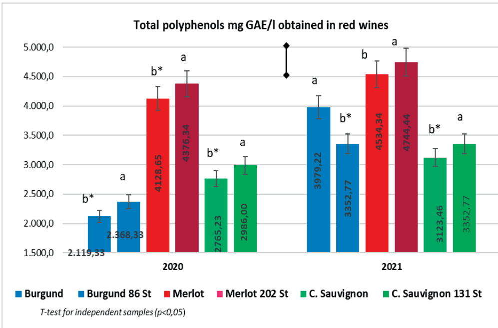

Figure 2. Content of experimental red wines in total polyphenols from 'Burgun', 'Cabernet Sauvignon' and 'Merlot' control cvs. compared to clonal selections

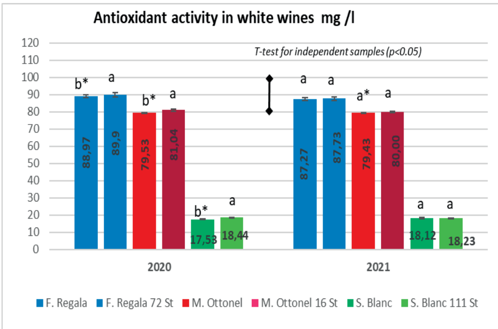

Figure 3. Content of experimental white wines in antioxidant activity from 'Feteasca regala', 'Muscat Ottonel' and 'Sauvignon Blanc' control varieties compared to clonal selections

The highest values regarding the concentration of wines in anthocyanins and polyphenols were evident in the clonal selection 'Merlot 202 St' followed by the reference cultivar. Even though the concentrations of these components in grapes can be affected by environmental conditions and the culture technology applied (Jackson and Lombard, 1993), the ratios between anthocyanin components and aromas are controlled by genotype.

'Cabernet Sauvignon' is one of the most widespread wine cultivars grown in Romania. In both experimental years the wines obtained from 'Cabernet Sauvignon 131 St' clone were highlighted by a higher content in total anthocyanins (they varied between 278.32 g/l in 2020 and 298.8 g/l in 2021) (Figure 4) and total polyphenols (2986.0 and 3352.77 mg GAE/l) (Figure 2) compared to the cultivar control. Similar results regarding the amounts of total anthocyanins in wine samples obtained from clone 54 of the 'Cabernet Sauvignon' cv. (255 g/l total anthocyanins) were also reported by Filip, 2018, in a study evaluating 3 'Cabernet Sauvignon' clones from the Valea Calugareasca area.

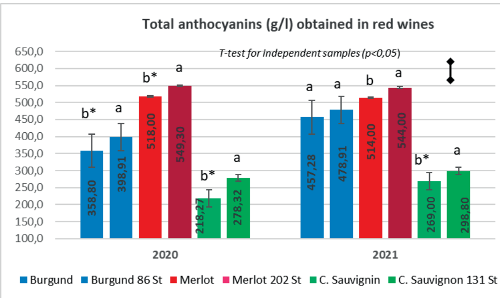

Figure 4. Content of experimental red wines in total anthocyanins from 'Burgun', 'Cabernet Sauvignon' and 'Merlot' control cvs. compared to clonal selections

CONCLUSIONS

The suitable wines from the control varieties 'Fetească Regală', 'Muscat Ottonel' and 'Sauvignon Blanc' but also their clonal selection have acquired an alcoholic strength of over 12.0% vol. reaching up to 13.73% ('Fetească Regală 72 St'), in the year 2020 being able to recommend to be marketed as superior quality wines, according to the legislation in force.

The amounts of total polyphenols recorded higher values in the wines obtained from the clonal selections 'Fetească Regală 72 St', 'Muscat Ottonel 16 St', and 'Sauvignon Blanc 111 St' (470.90 mg GAE/l polyphenols, 449.3 mg GAE/l, respectively 418.32 mg GAE/l), compared to those obtained from the reference cultivars 'Feteasca Regală', 'Muscat Ottonel' and 'Sauvignon Blanc', in 2020.

The red wines obtained in the Stefanesti culture area are appreciated as highly extractive wines. This fact is confirmed by the results recorded at NRDIBH Stefanesti, where non-reducing extract values of over 19 g/l, were obtained in the 'Burgund 86 St' clonal selection (between 21.82 g/l and 26.02 g/l in the two years study), and 'Cabernet Sauvignon 131 St' (between 29.2 g/l in 2020 and, respectively, 26.85 g/l in 2021). These values ensure the wine's consistency and personality.

Among the clones for red wine, we emphasize the clone 'Merlot 202 St' and the reference variety from which it comes, which recorded the highest values of total anthocyanins and polyphenols.

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VEGETABLE GROWING

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STUDY REGARDING THE INFLUENCE OF SOME CLIMATIC PARAMETERS FROM THE GREENHOUSE ON THE TOMATO PRODUCTION AND FRUITS QUALITY

Adnan ARSHAD, Ionuț Ovidiu JERCA, Sovorn CHAN, Sorin Mihai CÎMPEANU, Răzvan Ionuț TEODORESCU, Jeni ȚIU, Liliana BĂDULESCU, Elena Maria DRĂGHICI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: draghiciem@yahoo.com

Abstract

Greenhouses provide a controlled environment where temperature, humidity, and light can be optimized for tomato growth. The present study was carried out to estimate the growth, biochemical, and physiological responses of cherry tomatoes to some environmental conditions. Data was collected on different dates during the period extending from December 2022 to March 2023. Based on collected data, the potential growth rate of plants has been predicted: plant height, 2.43 cm/day to 2.98 cm/day; leaf growth, 0017 cm/day; three leaves per week. Plants with a high day length were high in height and produced more leaves. A significant relationship found between the inflorescence and the data collected for total mass/inflorescence, average size of fruits/inflorescence, diameter of fruit/inflorescence, and fruit height/inflorescence. A non-significant interaction found between the fruit keeping quality, fruit size, and inflorescence number. Regarding their biochemical responses, every inflorescence differs significantly from the others. A non-significant relationship was also found between fruit acidity and inflorescence order. The current study confirms a direct relationship between biochemical, growth, and productivity indicator under greenhouse conditions. An analysis of variance was used to statistically assess the collected data at a confidence level of p 0.05.

***Key words**: tomatoes, greenhouse, productivity, growth rate, biochemical attributes.*

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.), a south American-originating species, is the most widely cultivated vegetable in the world (Ohashi et al., 2022). Along with all types of peppers, potatoes, eggplant, and ground cherries, it is a major crop in the family Solanaceae. It is a well-known vegetable or fruit due to its nutritional and medicinal attributes, as it contains different types of minerals, vitamins, and antioxidants (Knapp and Peralta, 2016; Fatima et al., 2009). In view of its industrial importance, the tomato industry has been declared the most developed, geographically connected, and inventive horticulture industry (Costa and Heuvelink, 2007). It has been reported that 186.821 million metric tons of tomatoes were produced in the world during the year 2020 on 5,051,983 hectares, yielding an average of 37.1 metric tons per hectare (Branthôme, 2020).

Today, the world has been facing a constant threat of food scarcity and malnutrition, with the number of hungry people rising to 828 million in 2021, an increase of around 46 million since 2020 and 150 million since 1990 (WHO, 2015). It has now become the need of the hour for the scientific community to introduce innovative, time-consuming, and reliable methods of food production. Greenhouse is a Optimistic and sustainable method for the production of vegetables, especially tomatoes at large scale (Ofori et al., 2022). It has been widely adopted as an arbitrary and yield-oriented configuration in which growers can set desired environmental conditions (Taki and Yildizhan, 2018). People all over the world are replacing manual and traditional methods with advanced technology-based methods, and greenhouse technology could be a reliable alternative to land (Ohashi et al., 2022). There are plenty of studies that explain the behavior of greenhouses (Shimizu, 2007).

Previously, different crops were grown to observe the response of various variables, and a significant relationship between greenhouse conditions and plant morphological, biochemical, and growth parameters has been reported (Chang et al., 2013; Chan et al., 2022; Arshad, 2021).

A research report ranked the tomato as the second-most important vegetable crop in the world and reported it as an important component of the Mediterranean diet both quantitatively and qualitatively (Viuda-Martos et al., 2014). Plants adapt morphologically or functionally in response to their environment (Koning, 2000). To evaluate the response of tomatoes to different greenhouse conditions, different experiments were performed in the past (Draghici et al., 2021). Alsadon et al. (2018) found a direct relationship between greenhouse conditions and tomato plant growth and productivity. A greenhouse setup is a flexible solution for the cultivation of tomatoes, as it can provide optimally manageable growth conditions. Several growth phases and light conditions influenced the optimal microclimate conditions for the productive greenhouse cultivation of tomatoes. Garca et al. (2011) classified tomato growth into five phases: the initial leaf period, vegetative growth, flowering, early fruiting, and the mature fruiting period. The duration of time that each growing phase will last depends on the understudied tomato variety and growth control parameters: temperature, light, substrate, and nutrients. All the nutritional, structural, mechanical, and climate-based aspects of a greenhouse are crucial for plant growth, fruit quality, and production. (Shamshiri et al., 2018). According to a study, the growing periods of tomatoes grown under two greenhouse setups were 133 days and 126 days, respectively (Garcia et al., 2011). In a comparative study, De Gelder et al. (2002) reported a 9 percent increase in production, an 8-10 percent increase in dry matter, and high transpiration in a CO2- and air-circulated closed greenhouse compared to a normal, heated conventional greenhouse (Opdam et al., 2004). The influence of temperature on all aspects of tomato plant growth is significant. Plants grown under suboptimal temperature conditions produced thicker leaves and showed less growth. On the other hand, truss and leaf initiation are also directly influenced by temperature (Van Der Ploeg and Heuvelink, 2005). The optimal temperatures of 22-25°C for the development of leaves and trusses and 22-26°C and 22-25°C for fruit addition and fruit set, respectively (Sato et al., 2000). The percentage of humidity is another factor that can affect the growth of tomato plants to a great extent. The percentage of humidity is another factor that can affect the growth of tomato plants to a great extent. The relationship between the humidity percentage and temperature influenced the transpiration rate of plants (Kittas et al., 2005). ASABE (2015) recommended an average percentage of 60–90% of relative humidity for the growth of most tomato varieties in greenhouses. The spacing between the plants was reported as an important factor for plant yield per unit area. The spacing between the plants determines the density of plant leaves, which, alternatively, affects canopy light interception in tomatoes (Kaneko et al., 2015).

The production of vegetables in a controlled environment is highly advantageous, especially in cold regions. It is also important to note that some threats are also associated with this technology. Because greenhouse cultivation necessitates a significant initial investment in labor, equipment, and time, the grower must be aware of its advanced techniques in order to achieve a high yield at a low cost. In view of lowering input costs, greenhouse irrigation and fertilizer management can improve yield, conserve water, and improve product quality (Wang et al., 2022). The controlled environment can help the grower protect the plant from the attack of different diseases and also extend the production season. Though many unknown facts about greenhouse management and greenhouse benefits have already been reported, there are still many findings that require further investigation. In line with the previous idea, the present study was conducted to report the response of a local tomato variety to different greenhouse conditions. The study was based on the hypothesis that variation in green house conditions and green management will directly effect plant growth attributes, productivity, and fruit quality.

MATERIALS AND METHODS

A systematic and controlled approach experiment was carried out on tomatoes plants.
to observe and measure specific variables to understand their response to different greenhouse conditions in view on plant growth development and fruit production performance.
The studies were carried out on the premises of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV), at the Research Greenhouse and the Research Center for Studies of Food and Agricultural Product Quality. A Dutch hybrid Cherry tomato (*Lycopersicon esculentum* Mill.) cv. 'Cheramy F_1' seeds were sown in coconut substrate at the end of October 2022. Later on, 40 days old seedlings with first inflorescence were transplanted to coconut-containing growbag (Jiffy growbag), in a separate greenhouse compartment (Figure 1). The plants were appropriately prepared by providing optimal conditions for growth before the start of the experiment.
Tomatoes plants of similar size and physical appearance were randomly selected from each side (front, back, middle, left and right) of the greenhouse experimental compartment. All the Agricultural management practices (fertilizing, watering, and fungicide spray) were performed as per the recommendations. plant growth parameters and productivity were estimated during the period extended from 21st December 2022 to 3rd April 2023 (Table 1.). Plant growth parameters were observed on different dates with different days intervals to estimate the change in growth rate. Growth parameters included total number of leaves/plant, plant height, stem diameter, internodal branch distance. It has been reported that the internode branch distance is a proximate of plant's efficiency. Change in plant height, change in leaf growth rate was also estimated over the observation period. All the measurements were conducted using measuring tape with centimeter marking on it. To measure leaf growth, three leaves from each plant were selected and measured maximum length, distance from the base of the petiole to the distal end (Ls) and maximum width, farthest distance across the petiole (L+w) of each marked leaf were recorded. Evaluation of plant productivity was based on comprehensive assessment of productivity parameters, type of inflorescence, total number of inflorescence/ plants, length of inflorescence, number of fruits/inflorescences, total fruit mass/inflorescence, average fruit mass/Inflorescence, fruit height and circumference. The first seven inflorescences of each plant were observed. When each plant reached the stage of bearing their first eleven inflorescence were harvested to account the plant productivity and fruits physical attributes. Laboratory analysis includes fruit firmness, fruit acidity, fruit dry matter, brix (%) and nitrate contents/inflorescence (Figure 2). Fruit keeping quality was assessed by counting the number of days, fruits from each inflorescence survived the room temperature (whenever the wrinkle appears, and fruit skin becomes softer) in polythene zip bags.

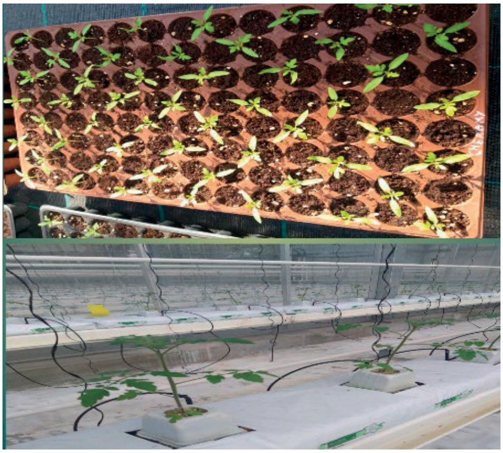

Figure1. Tomatoes plants at seedling and early growth stage

Table 1. Observations dates and days interval

Sr#	Dates	Days interval
1	21/12/2022	
2	6/1/2023	15
3	13/01/2023	6
4	20/01/2023	6
5	27/01/2023	6
6	3/2/2023	6
7	13/2/2023	10
8	2/3/2023	16
9	27/3/23	25

The graphs related to growth parameters were produced by using MS word and Jamvoi Software. The analysis of variance (ANOVA) was performed.with the subsequent use of the Normality Test (Shapiro-Wilk), Homogeneity of Variances Test (Levene's), at a confidence level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Data regarding the microclimate variables, Temperature, CO_2 and light was sampled during the experimental period extended between October 2022 to March 2023. Figure 3 displays the average, maximum and minimum temperature records, while Figure 4 depicts the average, CO_2 and light data for each month. A direct relation was observed between the microclimatic conditions and plants' growth and fruiting stages. Extreme changes were observed in environmental conditions. The average temperature during the vegetative growth and flowering stage was 21.20ºC and maximum and minimum temperature was observed 25.47 ºC and 16.94 ºC respectively.

The average light amount and, CO_2 concentration during the vegetative growth and flowering stage, was 349.19 (ppm) and 240.93 (w/m²). respectively was recorded. The growth and development of different crops in Greenhouse is directly influenced by the time duration of winter season and availability of light, CO_2 and temperature.

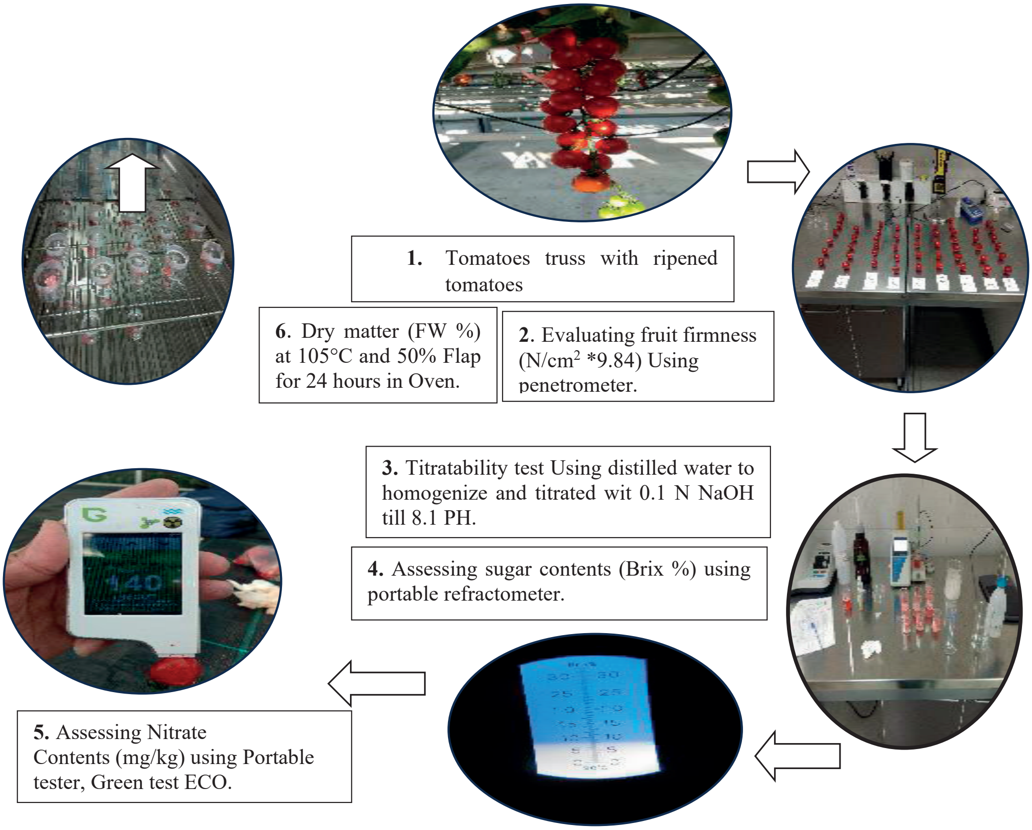

Figure 2. Laboratory analysis outline

Short-term light exposure in winter affects the growth and biochemical parameters of plants (Blom and Ingratta, 1984; Frantz et al., 2000). Plants physiological, metabolic, and developmental process are organized by endogenous biological clocks Müller et al., 2016; Thus, timely supplementation of light to plants can increase the yield and quality of fruits. Present study was also conducted in winter season under normal sunlight conditions. A significant interaction between the light duration and the concentration of different biochemical components was observed. Green house conditions have significant impacts on plant growth parameters (Katsoulas and Kittas, 2008).

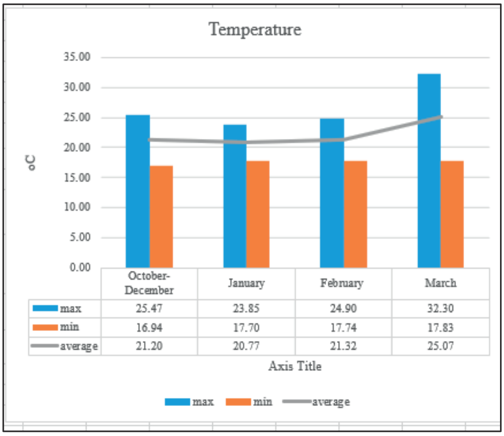

Figure 3. Monthly, average, maximum and minimum temperature ºC data

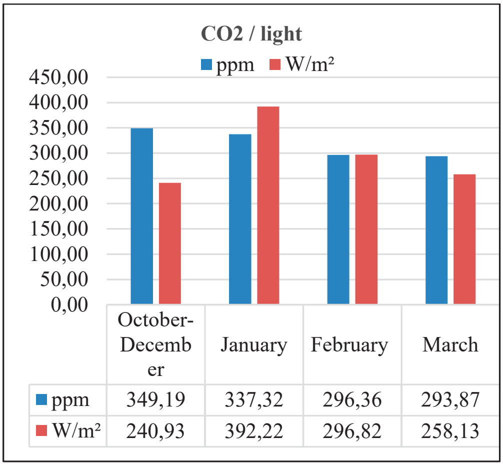

Figure 4. Average monthly data of CO_2 (ppm) & Light (w/m²)

The growth and development of different crops in Greenhouse is directly influenced by the time duration of winter season and availability of light, CO_2 and temperature. Short-term light exposure in winter affects the growth and biochemical parameters of plants (Blom and Ingratta, 1984; Frantz et al., 2000). Plants physiological, metabolic, and developmental process are organized by endogenous biological clocks (Müller et al., 2016). Thus, timely supplementation of light to plants can increase the yield and quality of fruits. Present study was also conducted in winter season under normal sunlight conditions. A significant interaction between the light duration and the concentration of different biochemical components was observed. Green house conditions have significant impacts on plant growth parameters (Katsoulas and Kittas, 2008). It has been observed that plants growth patterns have direct relation with temperature, CO_2 and light (Wheeler et al., 1990). In present study the growth and development stages of tomato plants were observed. The substantial increase in growth-related characteristics, such as total number of leaves/plants, total number of Inflorescence/plants, plant length (height), and dry weights, led to the highest overall yield. Efficiency index of tomato plants growth which decides the ability of plants to produce new material (productivity) was assessed. The results of growth parameters are mentioned in Table 2. Figures 5 and 6, presents the leaves growth rate. The data collected has showed that mature young (middle leaf) and newborn (upper leaf) significantly ($P = 0.002$, $P < 0.001$, respectively) differed from the old (base leaf) in view to observation on different dates.

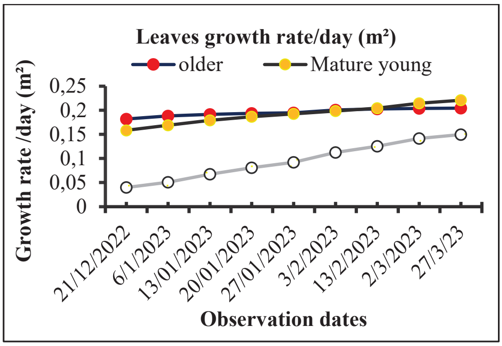

Figure 5. Leaves growth rate/day (m²)

The older leaves when reached at maximum growth stage; they stopped growing. The average growth rate of older and middle leaves was recorded (*.00034 cm/day and .00088 cm/day respectively*). The newborn leaf because of its position at top receive maximum amount of light as a result showed maximum growth per/day (*.0017cm/day*). Carmassi et al. (2007) reported a significant relation between the length of leaf and leaf position. It has also been reported that cultural practices and genetic diversity may affect the leaf morphology and leaf length Rouphael et al. (2006). Leaf growth rate was observed high in the month of March and low at the end of January (Figure 6). The change in leaf growth directly influenced by the environmental conditions (Zhang and Xiao, 2015).

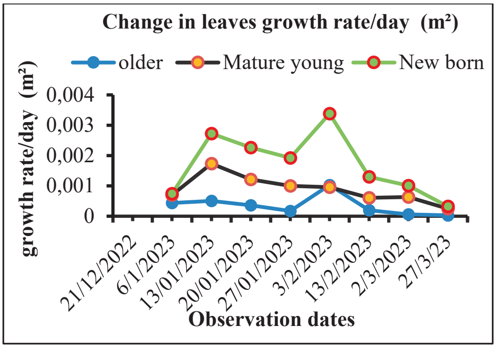

Figure 6. Change in leaves growth rate/day (m²)

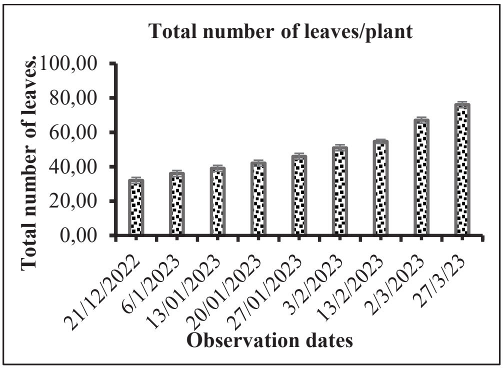

Figure 7. Total number of leaves/plants

In current time when this world has been battling with food challenges it is very important to understand the agricultural practices and scientific understanding. Growing plants in greenhouse conditions has many limitations. Plant height is a very important vegetative factor which responds directly to greenhouse conditions, temperature, humidity, carbon dioxide concentration and heat amount. Abdelmageed et al. (2003) reported a systematic and consistent difference among the tomato's plants height in response to their exposure to different heat shock treatment. Present study evaluated similar results. Figure 8 depicted the total height of plant during the observation period (December to March). A significant difference (*P<0.001)* has been found among the data collected on different dates (Table.2). It has been observed that the plant height growth rate per day drooped in the middle of February and high at the start of March (2.43 cm/day and 2.98 cm/day respectively). It was observed that average increase in plant height was within the range of 21 to 28 cm.

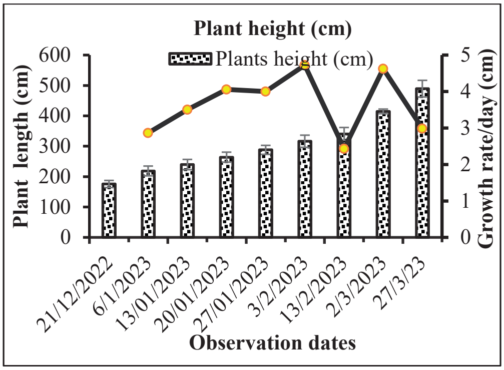

Figure 8. Plant height (cm)

There are different factors which affect the plant length (height). Rangaswamy et al. (2021) reported that high concentration of carbon dioxide increases the plant height, compared to plant grown in ambient carbon dioxide concentration. Plant height along with number of leaves have the potential to decides the future biomass production of plant. Plant height can influence the amount of light intercepted by leaves. Photosynthesis process is the driving force which increases the plant biomass. Biomass is integral component which decides the future plant productivity and overall yield. It has been reported, plant with higher number of leaves exposed to high concentration of carbon dioxide and temperature enhanced photosynthetic rate and water use efficiency (Jones, 2013). In present study findings a significant interaction (*P<0.001)* between total number of leaves and total height of plant has been observed. It was identified that number of leaves gradually increased, on average three new leaves formed every week and. In line with these findings Jo and Shin (2020) reported similar results in their comparison based study on two tomato cultivars under greenhouse conditions. There is no significant relation found between the plant heights and total number of leaves on second observation date. It was found that, Plants at the end of each row with more day length (remained exposed to daylight for maximum time) were high in height and produced a greater number of leaves (Figure 7). These results are consistent with the previous findings (Suyanto et al., 2012) which revealed that tomato plant growth rate (1.11 cm/day) is high at germination stage under the 680 nm, light compared other low wavelength light (480, 550, 650). Plants' growth rate vary from variety to variety under different growing conditions. The number of inflorescences significantly (*P<0.001*) vary between the plants on each observation date. Plants with high height produced more inflorescence compared to plants which has low growth rate. A plant with good growing condition has produced one Inflorescence per week. Adams et al. (2001) reported that temperature directly affects the number of inflorescences, high temperature causes early initiation of truss along with increase in fruit volume and size.

In terms of plant productivity, a significant relation between each inflorescence and total mass/inflorescence, average size of fruits/inflorescence, diameter of fruit/inflorescence, fruit height/inflorescence has been found. Number of fruits and fruit keeping days was not significantly affected by inflorescence number (*P = 0.176, P = 0.156, respectively*). A significant relation (*P = 0.026)* was found between total mass production and Inflorescence number. Total mass/inflorescence (expressed in grams) varied from inflorescence to inflorescence (Table 3). Inflorescence number 6 has produced maximum mass followed by inflorescence 7 and 1. Different greenhouse factors contribute in productivity of tomatoes plants. Plant day length is a key factor which directly influence the plant productivity. Alia et al. (2020) declared six hours lighting, a minimum time duration for fruit setting in tomatoes plants. Shading is another important factor which increases the productivity of tomato plants El-Aidy's (1983). Fruit mass is another parameter which help the grower to estimate the productivity tomato plants. Present study found a significant relation (*P = 0.021)* between average fruit mass and inflorescence number.

A study mentioned fruit mass a important indicator of plant productivity (Bădulescu et al., 2020). It was found that fruits from lower inflorescence have small size and low weight. Availability of carbon dioxide, Light, water and growing conditions ensure the magnitude of tomato plants productivity. Vanthoor et al. (2011) reported that average low temperatures treatment combined low light increases the productivity of tomato plant. Each part of plant receives different amount of light and other essential elements, which effect their productivity status. Moreover, increase in size of plant canopy also effect the growth of leaves and inflorescence at lower position (Sarlikioti et al., 2011). Present study has witnessed a interesting pattern in inflorescence morphology and length. Mostly inflorescences were single branched but bifurcated and trifurcated inflorescences also found. Inflorescence number three and six were bifurcated on each observed plant. Morphological nature of inflorescence plays a important role in the prediction of plant productivity.

A significant difference (*P<0.001)* found between the inflorescence length and number of inflorescences. A direct relation found between the length of Inflorescence and order of Inflorescence (Table.3) Inflorescence close to plant root (Lower Inflorescence) was small in length as compared to inflorescence above it and so on. Availability of quality water, following the process of transpiration help in moving the essential nutrients from base to metabolically active parts, leaves and inflorescence. This increases the growth rate in leaves and reproductive part and resultantly increase their length (Wei, 2017). There was no significant (*P = 0.176)* relation found between number of fruits and inflorescence number. Inflorescence number six followed by number seven and three has produced maximum number of fruits (Table 3). Previously it was discovered that as air temperature rose from 25 to 29°C, fruit number, the percentage of fruit set, and fruit weight per plant all dropped. (Shamshir et al., 2018). A study's findings reported that the number of fruits/inflorescences is directly influenced by daylight; more sunshine means more fruits will produce. Plant Fruits diameter vary significantly (*P = 0.031)* from inflorescence to inflorescence. Inflorescence number 4th followed by 3rd and 2nd appeared with high fruit diameter (Table 3). It has been previously reported that agricultural practices and treatment of different fertilizer increases the fruit size and production rate.

The complimentary actions of phosphorus and potassium, which aid in the synthesis of auxins, may also contribute to the increase in length and size of the fruits (Mudasir et al., 2009). A highly significant interactions (*P<0.001)* found between fruit dry matter and inflorescence number (Figure 9).

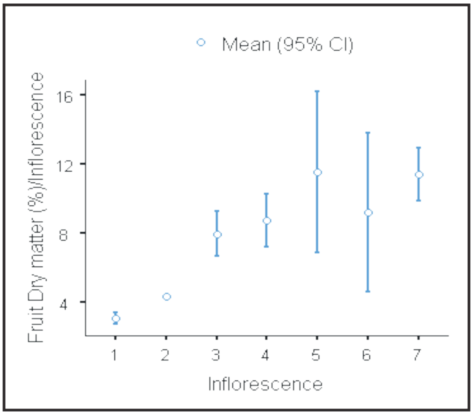

Figure 9. Fruit Dry matter (%)/Inflorescence

Inflorescence number 5 appeared with maximum fruit dry matter followed by 7th and 8th (Table 3). A study reported 7-8% increase in fruit dry matter in CO_2 treated tomato plants compared to tomato grown under conventional method (Opdam et al., 2004). Fruit firmness (N/cm^2) and its keeping quality are key attributes which decide the market value of tomato. More number of days a fruit can survive at room temperature the more it has market value. A significant interaction (*P = 0.061)* was found between fruit firmness and Inflorescence order. The 3rd inflorescence followed by 1st and 4th showed maximum resistance to applied force of penetrometer (8 mm probes). It was found that lower inflorescence fruits appeared with high fruit firmness compared to upper inflorescence (Figure 12). The reason behind this may be the physiological and biochemical activities as upper fruits branches receive maximum amount of light and key elements of the process of photosynthesis. Tadesse et al. (2015) reported that fruit anatomy and chemical composition directly affect the fruit firmness quality.

Plants physiological, metabolic, and developmental process are organized by endogenous biological clocks (Müller et al., 2016). Figures 10 and 11, significantly different concentration of nitrate (mg/kg) and Brix (%) found in all the observed inflorescence (*P = 0.010, P = 0.062, respectively)*. Following the old pattern lower inflorescence appeared low in nitrate and sugar contents compared to upper (higher) Inflorescence (Table 4).

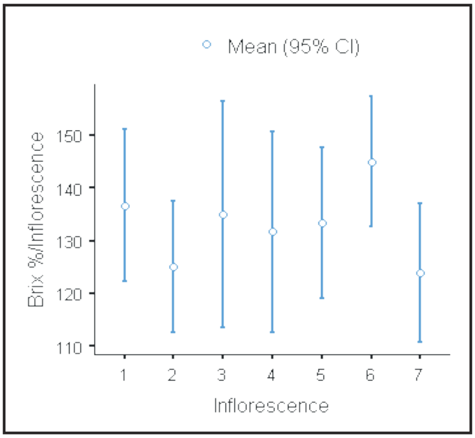

Figure 10. Brix (%)/Inflorescence

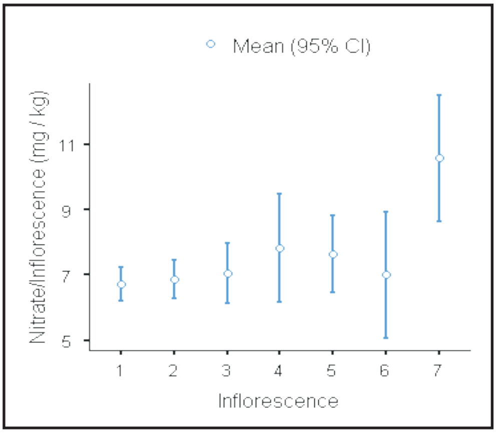

Figure 11. Nitrate contents/inflorescence (mg/kg)

The accumulation of sugar contents in the edible parts of vegetables is the result of CO_2

fixation under elevated conditions. Thus, timely supplementation of light to plants can increase the yield and quality of fruits. Previously, a non-significant interaction between the greenhouse conditions and the concentration of total titratable acidity in tomato plants was reported (Dong et al., 2014). Like this, present findings also determined a non-significant (*P=0.110)* interaction between the fruit acidity and inflorescence number. Inflorescence number three followed by 2nd and 1st show maximum value for fruit acidity (Figure 13).

Table 2. Growth parameters (Means ± SD) response to greenhouse conditions in tomato plants

Dates	Lower Older leaf	Middle Younger leaf	Upper Newborn leaf	Height of plant	Total number of inflorescences	Total number of leaves/ plants
	Mean, SD.	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**
21-12-2022	41.73±1.12	34.30±3.30	24.47±0.92	175.67±11.71	8.33±0.57	32.00±1.73
06-01-2023	42.07±1.13	35.90±1.55	26.23±1.07	218.67±16.07	9.33±0.57	36.00±1.73
13-01-2023	42.30±1.17	37.57±0.75	30.03±1.45	239.67±16.86	10.33±0.57	39.00±1.73
20-01-2023	42.73±1.02	38.47±1.19	31.90±1.13	264.00±16.46	11.33±0.57	42.00±1.73
27-01-2023	43.30±0.36	39.33±0.98	33.13±1.51	288.00±14.79	12.33±0.57	46.00±1.73
03-02-2023	43.70±0.60	40.13±0.72	34.50±1.74	316.33±20.03	13.33±0.57	51.00±1.73
13-02-2023	43.70±1.21	41.07±0.14	36.20±1.99	340.67±21.03	16.33±0.57	54.67±1.15
2-03-2022	43.90±1.21	42.17±0.75	38.37±2.50	414.33±7.50	19.33±0.57	67.00±173
27-03-2022	43.90±1.21	42.63±0.73	39.67±1.19	489.33±27.46	20.33±0.57	76.00±173

Table 3. Productivity (Means ± SD) response to greenhouse conditions in tomato plants

Inflores cence	Total mass (g)	Average fruit mass (g)	Inflorescence Length (cm)	number of fruits	Fruit Dry matter (%)	Fruit firmness N/cm2)	Fruit diameter (%)	Fruit keeping days
	Mean, SD.	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**	**Mean, SD.**
1	197.39±67.65	11.83±0.29	20.13±1.30	12.33±1.52	3.11±0.12	6.56±0.59	27.93±1.60	17.33±2.08
2	166.98±5.99	13.64±1.22	24.67±4.72	13.00±1.00	4.34±0.03	6.21±0.41	28.50±1.10	16.00±1.73
3	203.40± 13.18	11.74±0.70	29.27±0.70	15.67±2.30	7.96±0.52	7.28±1.55	27.98±1.18	14.67±1.52
4	143.94±9.91	9.84±0.49	36.07±1.09	14.00±2.00	8.75±0.61	6.37±0.49	29.25±0.48	14.00±2.00
5	170.52±14.80	10.89±0.20	39.10±1.65	15.00±2.00	11.54±1.88	6.05±0.45	26.51±0.52	13.00±2.00
6	265.40±72.95	10.36±1.47	40.60±3.83	26.33±9.01	9.23±1.85	5.73±0.36	27.47±0.98	10.67±2.51
7	242.88±44.39	9.67±1.53	38.27±0.057	26.00±7.81	11.41±0.61	5.43±0.07	27.0±0.89	12.33±1.52

Table 4. Biochemical (Means ± SD) response to greenhouse conditions in tomato plants

Inflorescence	Fruit acidity	Fruit firmness (N/cm2)	Brix %/	Nitrate (mg / kg)
1	15.94 ± 0.27	6.56 ±059	136.67 ±5.77	6.73 ± 0.20
2	16.15± 0.28	6.21±0.41	125.00±5.00	6.87 ±0.23
3	16.47±0.33	7.28±1.5	135.00±8.66	7.05 ±0.37
4	15.35±3.02	6.37±0.49	131.67±7.63	7.84 ± 0.66
5	15.28±0.43	6.05±0.45	133.33±5.77	7.63 ±0.47
6	14.42±1.77	5.73±0.36	145.00±5.00	7.01 ± 0.77
7	14.12±1.01	5.43±0.07	124.00±5.29	10.58 ± 0.77

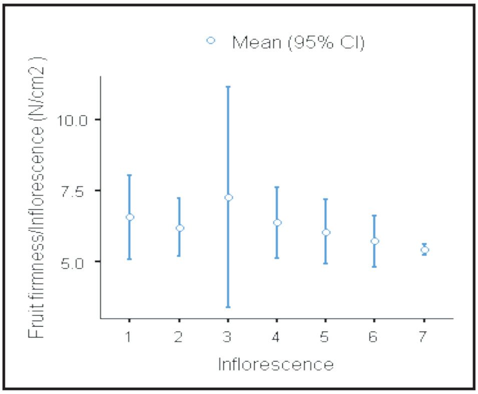

Figure 12. Fruit Firmness/Inflorescence (N/cm^2)

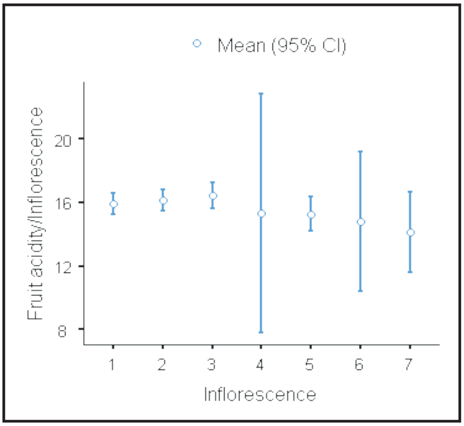

Figure 13. Fruit Acidity/Inflorescence

CONCLUSIONS

In present time, the world has been continuously battling against food scarcity problems. To meet food requirements, it is necessary to grow cereal crops and vegetables at large scale. Greenhouse is a advance method of growing vegetables and crops, which allow maintained the desired growing conditions. Tomato is one of the largest growing vegetables. It is a rich source of different minerals (potassium), vitamins (Vitamin C, Vitamin A, Vitamin K), and antioxidants. Greenhouse conditions, Temperature, humidity, Carbon dioxide and light directly influenced the growth and productivity of tomatoes. Applying growth promoting and productivity-oriented conditions one can grow tomatoes pant at large scale. Tomato as vegetable is considered as income-generating vegetable due to it high market value.

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REVIEW ON THE SUSTAINABILITY OF SOME REGENERATIVE AGRICULTURE PRACTICES FOR ORGANIC VEGETABLE GROWING

Dan Ioan AVASILOAIEI, Mariana CALARA, Marian Petre BREZEANU, Tina Oana CRISTEA

Vegetable Research and Development Station Bacau, 220 Calea Bârladului, Bacău, Romania

Corresponding author email: calaramariana@gmail.com

Abstract

Both the dynamics of world geopolitics and the environmental challenges rises a series of concerns for agriculture, in general, and vegetable growing, in particular, especially regarding the price and the carbon footprint of the inputs that are used.
In this regard, the trend of applying technologies that promotes the existence of cohesion and harmony between the various technological links at farm level becomes obvious. Among these, especially in the last decade, a particular amplitude is manifested in terms of regenerative agriculture practices. The present paper aims to evaluate the degree of regenerative agriculture practices sustainability with direct applicability to the ecological vegetable cultivation, highlighting the analogy of the two agricultural systems. In this respect, a relevant number of studies that addressed the topics involved were assessed, trying to synthesize the conclusive results and also to draw some potential directions to be followed in the very near future.

Key words: *regenerative agriculture; carbon footprint; vegetable cultivation; technological links; soil organic matter.*

INTRODUCTION

The very essence of regenerative agriculture is represented by the purpose of improving soil health and restoring the highly degraded land, simultaneously with an enhancement of water quality, vegetation and land-productivity. The Rodale Institute (2014) provides one of the most complex definitions of regenerative farming, considering it ”a long-term, holistic design that attempts to grow as much food using as few resources as possible in a way that revitalises the soil rather than depleting it, while offering a solution to carbon sequestration”. Shifting to regenerative agriculture practices also implies the uptake of a series of organic farming techniques designed to preserve and grow the quantity of soil organic matter, such as minimum tillage, cover crops and green manures cultivation, composting, mulching and crop rotation (Rhodes, 2017).
The awareness of a paradigm shift regarding conventional farming practices first occurred due to the event known in history as the Dust Bowl, generated by the land management practices deficiency in the US Great Plains region, enhancing its susceptibility before the 1930s drought. The extreme soil erosion emerged because of farmers abandoning soil conservation practices following the crop prices fall-off and high machinery costs, as well as turning into exploitation some inadequate lands for agriculture. Usually, the drought’s main effect is mentioned from an agricultural point of view. Several crops were affected by deficient rainfall, high temperatures and winds, insect infestations and dust storms. This situation facilitated the Great Depression’s bank closures, business losses, increased unemployment and other physical and emotional trauma. Moreover, the precipitation shortage would also have altered wildlife and plant life, generating water shortages for domestic needs.
A recent report on soil conservation, restoration, and improvement suggests taking a comprehensive approach to soil management known as Integrated Soil Fertility Management (ISFM). It involves incorporating organic matter such as crop residues and manure into the soil and cultivating legume crops like cowpeas that deposit nitrogen into the soil naturally.
Regenerative agriculture systems depend on the particularities of every socio-ecological and cultural context, where local and indigenous knowledge has a crucial function. In this approach, human beings are not detached from

nature, and tending for the environment represents a precondition for people caring (Anderson and Rivera-Ferre, 2021).

MATERIALS AND METHODS

Data source and selection criteria

Data were gathered from a comprehensive selection of scientific studies, primarily from the past two decades, that examined the benefits of implementing regenerative agricultural practices for organic vegetable cultivation. A total of 345 relevant papers were identified from databases such as Google Academic, ScienceDirect, and Springer.com, using search terms like "regenerative agriculture" "organic farming" and "recommended management practices". Only studies that met specific criteria were included in the analysis, such as being recent and immediately applicable, providing detailed information on the advantages of regenerative agriculture in organic farming and having relevant and sufficient research to draw conclusions from.

RESULTS AND DISCUSSIONS

Assessing differences between main farming systems

Usually, when referring to sustainability, the farming systems are divided in: organic (OFSs), integrated (IFS) and conventional farming systems (CFSs). While organic farming can be defined as "the science or art of managing/keeping under control agricultural organisms and their living environment for the long benefit of nature and humanity" (Toncea, 2002), the integrated farming system refers to "a holistic pattern of land use which integrates natural regulation processes with farming activities in order to maximize off-farm inputs replacement and sustain farm profitability" (El Titi, 1992; Morris and Winter, 1999; Pacini et al., 2003). On the other hand, the significance of conventional farming is often used in the literature to group a variety of practices that can be either more or less intensive.

Anderson & Rivera-Ferre (2021) provide a new perspective on the problem, labelling the agricultural systems on outcomes rather than practices, as follows: extractive and regenerative. Thus, a full comprehension of their characteristics would be obtained as opposed to a large debatable division in: sustainable agriculture, regenerative agriculture, climate-smart agriculture or agroecology, which retrives multiple forms of human and material capital in its focus on yields and profits (Gutierrez-Montes et al., 2009). Apart from providing food for human use, regenerative agricultural systems also sequester carbon, sustains biodiversity, offers diverse diets for malnutrition control, increases community well-being by maintaining farming livelihoods, support the dignity and autonomy of the person and mitigates external inputs and knowledge reliance (Anderson & Rivera-Ferre, 2021).

Finally, as shown in Table 1, Neiger (2019) proposes the following classification of agricultural system function dependent:

Table 1. Different types of agricultural systems (by Neiger, 2019)

Agricultural system type	Characteristics
SUSTAINABLE	It functions at a regular state without decreasing its long term capacity to operate
RESILIENT	It is able to regain its key functions after a disruption.
REGENERATIVE	It is flexible and increases operational capacity overtime; it has a positive effect on other systems

(https://www.regenerativedesigngroup.com/restoring-land-with-regenerative-agriculture/)

Carbon sequestration and GHG mitigation potential of some regenerative farming methods

Carbon stockpiled at soil level represents the largest terrestrial carbon pool. It is also 3.3 times the size of the atmospheric pool (760 Gt) and 4.5 times the size of the biotic one (560 Gt). The predominant range of soil organic carbon pool to 1 m depth is between 50 and 150 tons/ha, representing a dynamic equilibrium of gains and losses (Lal, 2004). A negligible change in soil C content can disrupt the global climate (Luo et al., 2010; IPCC, 2014). Vegetable cultivated soils are usually characterized by low soil organic carbon compared to permanent plant cover ones, where the values are significant higher. Thus, Jarecki and Lal (2003) showed that over the past 200 years, reconverting the natural land to agricultural use generated a loss between 50-100 Pg of soil organic carbon worldwide.

Similarly, Gelaw et al. (2014) and Wang et al. (2016) highlighted that land use/cover changes, especially agricultural activities, significantly affect ecosystem services including soil organic carbon (SOC) storage. At least temporarily, by the means of some recommended management practices, carbon stocks of these soils can be restored, thus removing CO_2 from the atmosphere. Nonetheless, up-to-date estimations of the actual soil C sink capacity are only 50-66% of the cumulative historic C loss (Lal, 2004).

An accurate description of the Carbon sequestration potential in world soils by adopting regenerative farming practices is presented in Table 2.

Even though the potential of SOC sequestration is finite (Lal, 2004b), it still has the capacity to offset between 5 and 15% of the global fossil-fuel emission (Kauppi and Sedjo, 2001; Lal, 2004b).

Stockmann et al. (2013) emphasizes the importance of the C dynamics understanding within agro-ecosystems and identification of appropriate farming practices in order to protect soil resources and provide adequate food and fiber for an ever-increasing population.

Therefore, soil represents a major influencer of the global carbon and nutrient cycle, holding more carbon than all terrestrial vegetation combined. Kopittke et al. (2019) showed that the use of soils for food production causes 30-60% of carbon loss, triggering the soil functionality decline. A global soil organic carbon map is presented in Figure 1.

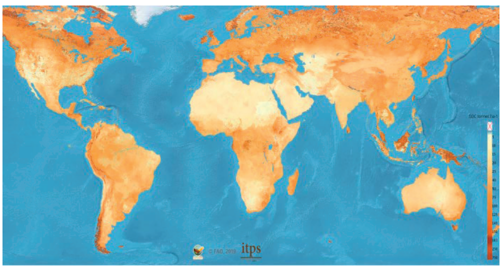

Figure 1. Global Soil organic Carbon Map (Scale 5-750 tons*ha^{-1}) (GLOSIS - GSOCmap ©FAO 2018, http://54.229.242.119/GSOCmap/)

Furthermore, the difference between the two layers (the one formed by areas where soil organic carbon is dominant and the one formed by the areas where biomass carbon is proeminent) is presented in Figure 2.

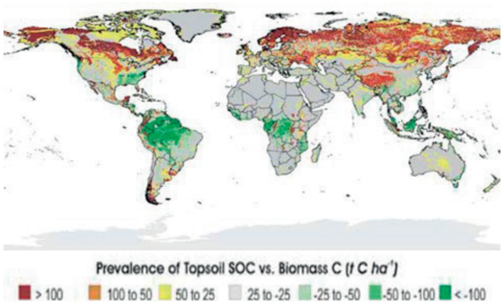

Figure 2. Prevalence of carbon in the topsoil or biomasshttps://www.fao.org/3/i5199e/i5199e.pdf

The assessment of agricultural impact on soil carbon sequestration emphasizes the carbon restore especially through animal manure recycling (Smith et al., 2001; Freibauer et al., 2004). While passing the digestive tract, manure is enriched in more sturdy compounds that can persist as stable soil organic matter in association with clay and silt particles. Above all, the application of composted manure has further advantages induced by the aerobic decomposition, where less CH_4 develops compared to stacked manure (Davis et al., 2002).

Several studies have evaluated the influence that irrigation (Houlbrooke et al., 2008; Kelliher et al., 2012), fertilization (Lemke et al., 2012; Yan et al., 2012), tillage (West and Post, 2002; Franzluebbers and Steiner, 2016) or land use change (Venkanna et al., 2014; Wiesmeier et al., 2015) has on soil organic carbon content and stocks in agricultural soils. Organic fertilizers substantially enhance soil C content as opposed to the chemical ones (Leifeld et al., 2009; Brar et al., 2013).

Regarding organic vegetable cultivation, Lal (2004), Liao et al. (2015) or Matsuura et al. (2018) emphasized the great potential of its practices to increase C stocks at soil level. By contrast, Leifeld and Fuhrer (2010) pointed out that the positive effects of organic system on SOC might be caused by the exceedingly applications of organic fertilizer compared with conventional system. In this respect, Powlson et al. (2011) consider that SOC increase due to organic fertilizer does not represent a genuine C sequestration.

As for agricultural GHG mitigation efforts, organic farming systems may be of paramount importance, because it uses less energy and stores more C per hectare than conventional system (Larsen et al., 2014; Reganold and Wachter, 2016). Meanwhile, on a production unit basis, both energy use and carbon footprint do not always favor organic (Meier et al., 2015; Reganold and Wachter, 2016).

Lal (2004) highlights the fact that beside increasing food security, carbon sequestration has the capability to offset fossil-fuel emissions by 0.4 to 1.2 gigatons of carbon per year, or 5 to 15% of the global fossil-fuel emissions (Lal, 2004). In this sense, the restoration of degraded soils and ecosystems whose resilience capacity is intact becomes essential (Silver et al., 2000).

Table 2. Potential of Carbon Sequestration in World Soils by adopting regenerative farming practices (Lal, 2004)

Cropland Soils: 1350 Mha [0.4 to 0.8 Gt C/yr]	**Irrigated Soils: 275 Mha [0.01 to 0.03 Gt C/yr]***	**Range Lands and Grass Lands: [0.01 to 0.3 Gt C/yr?]***	**Restoration of Degraded and Desertified Soils: 1.1 billion ha [0.2 to 0.4 Gt C/yr]**
Conservation tillage (100-1000)	Using drip/sub-irrigation	3.7 billion ha in semi-arid and sub-humid regions	Erosion control by water (100-200)
Cover crops (50-250)	Providing drainage (100-200)	Grazing management (50-150)	Erosion control by wind (50-100)
Manuring and Integrated Nutrient Management (50-150)	Controlling salinity (60-200)	Improved species (50-100)	Afforestation on marginal lands (50-300)
Diverse cropping systems (50-250)	Enhancing water use efficiency/water conservation (100-200)	Fire management (50-100)	Water conservation/harvesting (100-200)
Mixed farming (50-200)	Both soil organic and inorganic Carbon are sequestered	Nutrient managemen	
Agroforestry (100-200)		Both soil organic and inorganic Carbon are sequestered	
High potential for about 250 Mha in South America of acid savana soils			

Going regenerative in "4 per 1000" initiative context

The "4 per 1000" (4p1000) initiative has been launched during the COP 21 in Paris in 2015 and was based on transposing the science of soil carbon sequestration into action at the global scale. According to Lal (2020), the initiative represents an example of a broader set of negative emission technologies. The main features of the initiative are presented in table 3.

Better management practices have the ability of transforming agriculture from a net source of GHGs to an intense sink of atmospheric CO2 (Lal et al., 2018). By adopting this recommended management practices (RMPs) in a cost-effective manner, soil and biomass-C stocks and emission reductions can be measure and monitor. De Pinto et al. (2010) outlined the industry role in developing mechanisms in order to gather farmers in rural communities and design markets and contracts.

Furthermore, a 2018 study emphasized the role of governments and markets that needs to establish a baseline price levels and develop a methodology for carbon permits allocation and carbon finance initiatives to operate in a fair, just and transparent manner (BWP, 2018).

In order for carbon farming to be successful, carbon gains in agro-ecosystems (soil and biomass) through improved management must exceed the erosion, decomposition and harvest losses (Carbon Cycle Institut, 2020).

Also, the key of the 4p1000 initiative is represented by the creation and operationalization of carbon trading markets.

By the implementation of the essentially regenerative RMP (Recommended management practices) that sequester SOC and mitigate emissions, carbon markets can offer a new source of income for farmers (Koper, 2014; Gustin, 2017). Being scale-neutral, carbon farming feasibility for both small-scale and large-scale commercial farms is certain. Becker et al. (2013) emphasizes the prospect of climate change mitigation in hot and dry areas by adopting regenerative practice to sequester carbon at soil level.

Table 3. `4 Per 1000 Inititive` - the core of regenerative movement for the years to come

`4 Per 1000 Inititive`			
History	**Signification**	**Main implementation methods**	**Literature**
- has been drafted at 2015 Climate summit held in Paris with the 21st Session of the Conference of the Parties and the 11th Session of the Conference of the Parties serving as the meeting of the Parties to the Kyoto Protocol	- an annual soil carbon content increase of 0.4 percent on a 30-40 cm depth that will determine a major balance of the CO_2 triggered by human activities	- intercropping	Corbeels et al. (2018); Mikula et al. (2020)
		- improved crop rotations	Francaviglia et al. (2019); Wiesmeier et al. (2020); Bruni et al. (2021)
		- organic farming	Leu (2017); Garcia-Palacios et al. (2018); Keel et al. (2019); Wiesmeier et al. (2020)
		- agroforestry – woody plants (tree or shrubs) are mixed with vegetable crops	Arango-Quiroga (2019); Cardenas et al. (2017); Wiesmeier et al. (2020)
		- conversion of arable land to grassland	Soussana,et al. (2017); Rodrigues et al. (2021)

Best of regenerative farming practices to adopt in organic vegetable cultivation

Some of the best regenerative farming practices that are suitable for organic vegetable growing are presented in Table 4.

Using catch crops/cover crops will generate a permanent vegetal cover for land, extending the carbon assimilation period whilst preventing soil erosion, weeds infestation and nitrate losses (Poeplau and Don, 2015; Kanders et al., 2017; Strickland et al., 2019; Chahal et al., 2020). Legume varieties, several grasses and some cruciferous species are usually sown after the harvest of the main crop or undersown in/with main crops, being used as fodder crops for ruminants or as green manure, with soil improvement role (Lawson et al., 2015; Bleuler et al., 2017; Koehler-Cole and Elmore, 2020). Tiefenbacher et al. (2021) underline the positive soil organic carbon balance of utilizing catch crops in rotations.

Typically, the carbon sequestration potential of an annual catch crops cultivation was of 403 ± 142 kg C ha^{-1} y^{-1} in agricultural topsoils (0-25/30 cm) (Chambers et al., 2016; Bleuler et al., 2017; Jian et al., 2020). Likewise, Hu et al. (2018) emphasized an increase in topsoil organic carbon stocks (0-25 cm) of 210 kg C ha^{-1} y^{-1} due to the catch crops introduction into rotation. Furthermore, Jian et al. (2020)`s meta-analysis of 131 studies across the globe highlighted a mean carbon sequestration rate of 560 kg C ha^{-1} y^{-1}. Similarly, Bleuler et al. (2017) assessed cover cropping influence under permanent crops at a rate of 550 kg C ha^{-1} y^{-1}.

In organic vegetable fields, crop diversity can be enhanced on a temporal (crop rotation, catch crops) or spatial scale (several plant species at the same time, cover crop mixture). The variety of crop rotation and organic fertilizers/ amendments usage and/or perennial cropping systems have the capability of a better soil organic carbon storage compared with conventional (single) cropping systems (Minasny et al., 2017; Don et al., 2018), simultaneously enhancing soil microbial diversity, soil aggregate stability and subsoil organic carbon due to deep-rooting crops (Tiemann et al., 2015; Finney and Kaye, 2017). In terms of soil organic carbon storage, deep-rooting crops are determined, since roots retention is up to 2.3 times higher than the aboveground biomass (Kätterer et al., 2011; Gherardi and Sala, 2020). The positive effect prevails in the topsoil and declines with soil depth (Kaiser and Kalbitz, 2012). Börjesson et al. (2018) outline an enhancement of carbon sequestration potential by 360 and 590 kg C ha^{-1} y^{-1} in the topsoil (0-20 cm) at clay and, respectively, loam texture sites due to incorporating legumes in the rotation for 35 years.

Sokol et al. (2019) emphasize the deep-rooting crop species and varieties role of transferring carbon into the subsurface (where a high carbon sequestration potential exists) through root exudates (sugars, amino acids and other organic acids), particularly when organic substances are protected in organo-mineral aggregates (Paustian et al., 2016).

The deep-rooting crops cultivation can deliver a sequestration of 374 ± 117 kg C ha^{-1} y^{-1} (Börjesson et al., 2018; Poulton et al., 2018; Poffenbarger et al., 2020).

On the other hand, Lugato et al. (2018) highlight that carbon sequestration via N-fixing crops is limited to the first 20 years, thereafter, N_2O emissions exceeding the ability of these crops to mitigate CO_2 emissions.

Some extra benefits of deep-rooting crops are represented by their ability to use resources such as water and nutrients from the subsurface horizon, preventing nitrogen leaching and assuring a better plant resiliance to drought (Hansen et al., 2019). Also, they enhance deep infiltration and improve the pore connectivity of soils (Freibauer et al., 2004), augmenting the subsequent crops expansion throughout biopores.

Natural farming is another low-input regenerative method that uses weed residue mulching as an unique form of agroecosystem management to continuously increase soil carbon sequestration. Ultimately, it also reduces soil bulk density and enhances soil quality. Natural farming has the potential of making organic vegetable production compatible with environmental conservation. However, Dewi et al. (2022) warn about the importance of nutrient balance during long-term management in order to ensure that the necessary nutrients are available.

A series of authors emphasizes the use of biochar as an example of carbon farming solution to anthropogenic climate change, being an important negative emission technology (Smith, 2016; Jackson et al., 2017; Alcalde et al., 2018). It relies on implementing known and proven land use and soil management practices.

Organic matter ties the soil particles into aggregates, improving soil structure and infiltration rates while reducing compaction. It also run as a nutrients and water sink in the soil, as well as heightening microbial biodiversity and activity (Xu et al., 2022).

Usually, adding organic amendments or using them alongside cover cropping in mixtures could represent a feasible alternative for vegetable growers since these treatments showed beneficial effects on soil health (Baffaut et al., 2020; Conway et al., 2020; Xu et al., 2022).

Montgomery et al. (2022) highlighted that regenerative farm had almost three to four times the soil organic matter and a soil health score three to seven times higher compared to conventional farm.

Regarding the quality of the crop, cabbage grown in regenerative system had higher values for vitamin K (46%), vitamin E (31%), vitamin B_1 (33%), vitamin B3 (60%), vitamin B5 (23%), calcium (41%), potassium (22%) and less than a third of the sodium, 35% more carotenoids and 74% more phytosterols compared to cabbage from a regularly tilled organic field.

In addition, regenerative cultivated spinach presented a total phenolic content about 4 times higher compared to conventional system yield. Similarly, regenerative carrots had 60% to 70% more total phenolic content compared to conventional ones (Chun et al., 2005).

Table 4. Regenerative farming practices suitable for organic vegetable growing

Regenerative farming practices	Main features
Conservation cover	- a permanent vegetative cover; - plants that generates high volumes of organic matter in order to sequester carbon and enhance soil health are suitable;
Conservation crop rotation	- growing crops in a planned sequence on the same field over time;
Residue and Tillage Management, No-Till	- maintaining the preceding crop waste throughout the year and planting the subsequent crop directly into it;
Residue and Tillage Management, Reduced Till	- it limits soil-disturbing operations, expanding soil-carbon stocks and intensifying plant-available moisture;
Contour Buffer Strips	- narrow strips of continuous, herbaceous vegetative cover set on sloping cropland;

	- major role in reducing soil erosion and improvement of water quality and infiltration along with a stronger soil health;
Cover crops	- are set for a seasonal vegetative cover and consist of either legumes or grasses; - they lacks the cash crop role, being accountable for building soil structure and health by increasing organic matter and carbon stocks;
Field border	- a strip of permanent vegetation that encircles a cropland or it is placed at its edge;
Filter strips	- herbaceous vegetation with contaminants removal role from overland flow
Grassed waterways	- channels planted with grass and other suitable vegetation in order to reduce the water runoff speed;
Mulching	- use of plant waste or other materials to the land's surface; - enhances soil carbon sequestration and moisture management and reduces erosion;
Stripcropping	- use of a systematic arrangement of planned rotation crops that are erosion-resistant and erosion- susceptible on a cropland field
Vegetative barriers	- permanent strips of dense vegetation set in flow areas
Herbaceous wind barriers	- herbaceous vegetation set in narrow strips with role in wind speed and erosion mitigation

Permaculture - a state of the art way of growing organic vegetables by embracing regenerative principles

Permaculture is a low impact agricultural method that uses perennial cultivation methods to produce food crops through a series of principles that are in harmony with nature (Mollison & Jeeves, 1988; Holmgren, 2002; Rhodes, 2017). Land use in permaculture is closely linked with agroecology, agroforestry and traditional and indigenous practices. Two broad criteria are at the core of permaculture view: ecosystem mimicry and system optimization. Thus, it promotes some pragmatic methodological principles in order to develop resilient, autonomous and equitable living spaces. Both biodiversity and agrobiodiversity are valued for their positive effect on resilience: high-energy foods should consist in cereal crops, root vegetables and fruits from mini-orchards. Also, Morel et al. (2019) outline that the same element must fulfil multiple functions: e.g. a legume supplies of protein and improves the soil fertility. Therefore, the key principle of permaculture is the maximization of desirable connections between elements in order to achieve their best synergy and optimal design.

Another fundamental principle of permaculture is that the entity is more important than the sum of its parts. It requires an integrated 'systems thinking'. Permaculture design objective is to minimise waste, human labour and inputs of energy and other resources, establishing maximal benefits systems in order to fulfil a high level of holistic integrity and resilience. Hence, permaculture designs are 'organic' and grow over time according to the interplay of these relationships and elements having the potential to become extremely complex systems, able to produce a high density of food and materials with minimal input. Falk (2013) shows that a regenerative farm based on permaculture principles will develop an evolving ecological structure and biological production that increases in its complexity with time. Moreover, the overall biological yields will continue to grow, while the external inputs will decrease.

Rhodes (2017) outlines three ethical principles of permaculture design that are briefly presented in table 5.

Furthermore, Holmgren (2002) has identified twelve principles of permaculture design: (1) observe and interact, (2) catch and store energy, (3) obtain a yield, (4) apply self-regulation and accept feedback, (5) use and value renewable resources and services, (6) produce no waste, (7) design from patterns to details, (8) integrate rather than segregate, (9) use small and slow solutions, (10) use and value diversity, (11) use edges and value the marginal, (12) creatively use and respond to change.

Most of the goals of agricultural permaculture align with the aspirations and objectives of organic agriculture. However, unlike permaculture, the organic system adheres to well-defined regulations that enable expansion and replication. These rules are understandable to consumers. Conversely, several aspects of permaculture, such as the management of animal farming amendments or the usage of plant protection items, like neem oil or copper products, lack regulation, including related maximum restrictions. It is important to openly discuss with consumers, who expect safe products with clear knowledge of their origin and production methods, whether commercial permaculture is viable without organic agriculture standards. As long as there are no consistent and obligatory standards for permaculture, its implementation in commercial environments will likely remain debatable (Fiebrig et al., 2020).

The primary considerations for designing agroecosystems using permaculture techniques are (i) site characteristics; (ii) the interplay between various components across multiple levels, such as mixed crop cultivation at the plot level and diverse land utilization at the agro-ecosystem level; and (iii) the spatial configuration of the elements as crucial factors that impact multiple functions (Ferguson & Lovell, 2014; Holmgren, 2002). Permaculture has not originated most of the approaches it employs. Instead, it can be viewed as a conceptual framework for assessing and integrating pre-existing methods (Krebs & Bach, 2018).

Similar to organic and biodynamic agriculture, permaculture places significant emphasis on soil fertility. Permaculture shares many similarities with traditional organic farming, agroecology and biodynamic farming, in that all of these approaches advocate for a harmonious and respectful coexistence of humans and nature. However, biodynamic farming historically evolved from spiritual concerns (theosophy), while organic farming and agroecology are more closely linked to the collective and political struggles of peasants who fight for their autonomy. In contrast, permaculture emerged to support self-sufficient initiatives at an individual and community level, in preparation for a world less reliant on petrol.

As organic and biodynamic farming, permaculture attaches a great attention to soil fertility. Permaculture has much in common with traditional organic farming, agroecology, and biodynamic farming, in the sense that all these approaches promote a harmonious and respectful integration of human beings in nature. However, biodynamic farming has a historical association with spiritual concerns (theosophy), while organic farming and agroecology have stronger ties to peasant's movements collectively and politically fighting for their sovereignty, whereas permaculture was born to support individual and community-scale self-sufficiency initiatives in preparation for a post-petrol world.

The combination of management practices and the consequent characteristics of agroecosystems observed in permaculture farms are associated with a wide range of ecosystem functions and services (Hathaway, 2015; Krebs and Bach, 2018). Firstly, current research on perennialization indicates that the deliberate integration of perennial species can promote provisioning (agricultural yields), regulating (pest control, hydrological cycles, water quality, carbon sequestration, and storage), and supporting (soil quality, pollination) ecosystem services (Asbjornsen et al., 2013; Corry, 2016). Secondly, permaculture's emphasis on not just biodiversity but also enhancing yield through beneficial interactions may have anticipated the growth of the functional diversity field; modern ecologists refer to this as overyielding driven by complementarity or facilitation (Hooper et al., 2005; Szumigalski and Van Acker, 2005).

Ultimately, permaculture's groundbreaking idea that agricultural landscapes should strive to be diverse, varied, and incorporate areas for conservation (Mollison and Holmgren, 1978) anticipates modern wildlife-friendly matrix and agricultural mosaic models (Tscharntke et al., 2005; Kremen, 2015). Due to the uniform interpretation and implementation of permaculture principles among independent adopters and its extensive global recognition, permaculture is in a favorable position to impact the provision of numerous agroecosystem services.

In contrast, Ferguson & Lovell (2014) highlighted the downside of permaculture movement by the fact that starting from the founding parents, who had a solid, academic scientific background, here named Mollison, followed by his apprentice, Holmgren, gradually, the movement has become isolated from the scientific side, acquiring a pronounced empirical character In this regard, both Scott (2010) and Chalker-Scott (2010) emphasizes that Most permaculture texts do not refer to contemporary scientific research.

Table 5. Key ethics of permaculture (by Rhodes, 2017)

Permaculture ethics	Features
'Earth Care' (take care of the Earth)	Provision for all life systems to continue and proliferate.
	Work with nature
	Act to prevent damage and destruction
	Consider the choices we make
	Aim for minimal environmental impact
	Design healthy systems to meet our needs
'People Care' (take care of the people)	Supplying people`s access to the necessary resources for their existence
	Look after ourselves and others
	Working together
	Assist those in need of food and clean water
	Develop environmentally friendly lifestyles
	Design sustainable/regenerative systems
'Fair Shares' (share the surplus)	Healthy natural systems use outputs from each element to sustain others.
	Resources are limited and only by consumption mitigation durability should be achived
	Build economic alternative
	Develop a common unity
	Modify our way of life now in order to become part of the solution and not of the problem.
	Need to become reconnected with the natural world: shift in thinking and being.

CONCLUSIONS

Regenerative agriculture systems promote nutritious food, carbon sequestration, biodiversity conservation, community welfare improvement, and uphold human dignity and autonomy, while reducing reliance on external inputs and knowledge.

Recommended management practices for regenerative agriculture have the ability to convert agriculture from a net emitter of greenhouse gases to a strong absorber of atmospheric CO_2.

The regenerative agriculture techniques appropriate for cultivating organic vegetables include incorporating catch crops/cover crops, crop rotation and intercropping, natural farming techniques, mulching, and implementing reduced or no-till systems.

The utilization of land in permaculture is strongly associated with agroecology, agroforestry and traditional and indigenous methods, with the goal of enhancing its intricacy over time.

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INFLUENCES OF PLANT DENSITY AND SEEDLING PLANTING DATES ON CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA*) SEED PRODUCTION EFFICIENCY

Alexandru BUTE, Andreea ANTAL-TREMURICI, Creola BREZEANU, Silvica AMBĂRUȘ, Petre Marian BREZEANU, Gabriel-Alin IOSOB, Mariana CALARA

Vegetable Research and Development Station Bacău, 220 Calea Bârladului Street, Bacău, Romania

Corresponding author email: alexandru.bute@outlook.com

Abstract

Cabbage (Brassica oleracea var. capitata) is a prevalent vegetable crop cultivated for its nutritious leaves, which have a variety of culinary and medicinal use. As a consequence, vegetables such as cabbage are extremely popular among consumer demands. Constraints in the seed production chain highlight the significance of seed germination and crop`s growth at the farm level. The production of high-quality seeds is a crucial step in this crop`s cultivation since it impacts the success of germination and plant growth. In this study, we aimed to investigate the influence of plant density and seedling planting dates on cabbage seed process performance. This research is a component of a larger project aimed to devise a comprehensive and new modernized strategy for cabbage seed production. The experiment was carried out on `Silviana` autumn cabbage variety during two growing seasons, a field trial was undertaken to assess three plant densities (low, medium and high) and three seedling planting dates (early, mid-season and late). Cabbage seeds were also subjected to a range of seed viability and vigour tests.

***Key words**: seed yield, germination, Brassicaceae, cabbage crops, plant spacing.*

INTRODUCTION

Cabbage is a biennial cruciferous plant that produces a terminal head in the first season of growth and reproductive structures in the second season of growth.

Cabbage *(Brassica oleracea* var. *capitata*) is a leafy vegetable known for its high content of health-promoting bioactive compounds, including fiber, vitamins, glucosinolates, polyphenols, and flavonoids (Nawaz et al., 2018; Novotny et al., 2018; Haghighi et al., 2020). Flavonoids possess various beneficial properties, including anti-tumor, anti-aging, and anti-inflammatory effects, and can protect against diseases caused by free radicals generated by oxidative stress (Xiao et al., 2016). Vitamin C and polyphenols are the primary antioxidants that protect cells from oxidative damage by scavenging free radicals (Podsędek, 2007).

Sprouts represent the initial stage of seed germination and contain high levels of bioactive compounds, such as minerals, amino acids, vitamins, and flavonoids, compared to mature plants (Kim et al., 2004; Xu et al., 2020). Sprouts are also a rich source of dietary antioxidants (Bendary et al., 2013), and the bioactive compound content in sprouts is influenced by seed quality and developmental stage (Nam et al., 2018).

Seeds store all the nutrients required for plant growth, including vitamins, minerals, proteins, fats, and carbohydrates. Similar to other cruciferous crops, cabbage undergoes vernalization, which triggers flowering after exposure to cold temperatures (Miller, 1929). Due to a prolonged juvenile phase and strong vernalization dependence for flowering (Ito and Saito, 1961; Miller, 1929), cabbage requires at least six months, even under optimal conditions in a climate-controlled environment, to complete one generation. In extreme cases, some cabbage accessions may not flower even after overwintering (Kinoshita et al., 2021).

Various studies have identified genes that regulate the vernalization process in *B*. *oleracea*, with the major ones being homologs of *FLOWERING LOCUS C*, a master regulator of the vernalization floral pathway in *Arabidopsis thaliana* (Abuyusuf et al., 2019; Irwin et al., 2016; Lin et al., 2018; Michaels & Amasino, 1999; Okazaki et al., 2007). The strong vernalization requirement of cabbage offers a benefit in agricultural production as it can effectively suppress the incidence of bolting, a process marked by the elongation of stems followed by flowering, causing damage to the harvesting sites (Jung and Müller, 2009).

The planting date and the plant density are critical factors in the seed production practices of cabbage. Appropriate planting dates and optimal plant spacing play a significant role in determining yield-contributing characteristics and overall seed yield. Furthermore, these factors have a pronounced impact on the quality attributes of the harvested seeds. Therefore, selecting appropriate planting dates and optimal plant spacing can significantly improve the yield and quality of cabbage seed production.

Our study will contribute to the understanding of the factors that influence cabbage seed production efficiency and provide insights into optimal plant density and seedling planting dates for cabbage seed production. These findings will be valuable to farmers and seed companies seeking to produce high-quality cabbage seeds and meet the increasing demand for this nutritious vegetable crop.

Previous investigations, such as those conducted by Singh et al. (2010), Thirupal et al. (2014), and Jayamanne et al. (2015), have indicated that planting date has a significant impact on the vegetative and generative characteristics, head attributes, and seed yield of cabbage plants. This is due to the direct relationship between planting date, planting space and the maturity and harvesting time of cabbage, which is influenced by environmental factors such as temperature, day length, and light intensity. Choosing a suitable planting date is crucial for ensuring optimal growth conditions for cabbage plants and maximizing total and marketable yield, as observed in studies by Wszelaki & Kleinhenz (2003) and Tendaj and Sawicki (2012). These studies have demonstrated that earlier planting dates result in higher yields, while delayed planting leads to a decrease in both total and marketable yield.

Additionally, planting date affects the traits of the cabbage head and core traits, as evidenced by research conducted by Greenland et al. (2000), Orzolek et al. (2000), Kleinhenz and Wszelaki (2003), and Faizullah et al. (2015). Late planting dates were found to result in denser cabbage heads and head volume, while earlier planting dates produced heavier heads with larger diameters and wider core widths compared to late planting. Moreover, Faizullah et al. (2015) reported a reduction in head diameter and head weight with delayed planting dates.

In this study, we aim to investigate the influence of plant density and seedling planting dates on cabbage seed process performance. This research is part of a larger project aimed at developing a modernized strategy for cabbage seed production.

We conducted a field trial on the 'Silviana' late cabbage variety during two growing seasons to assess the impact of three plant densities (low, medium, and high) and three seedling planting dates (early, mid-season, and late) on cabbage seed production. We also evaluated the viability and vigor of cabbage seeds using established methods (ISTA, 2020).

The success of seed production in autumn cabbage is largely dependent on the crop's generative phase, which is a critical link in the seed production chain. It is well-established that the primary biological and physical characteristics of seeds are directly and significantly influenced by harvesting and conditioning operations, as well as by crop establishment techniques. These seed traits are the phenotypic expression of the cultivar, influenced by planting densities and the establishment period of the seed-producing crop. In this context, the research objective is to determine the extent to which planting date and density affect the quantity and quality of obtained seeds, and to identify the most efficient methods and techniques for maximizing the production of superior-quality seeds.

The overarching aim is to elucidate the interplay between crop establishment techniques and seed characteristics, and to optimize seed production through a comprehensive understanding of the underlying biological and environmental factors.

MATERIALS AND METHODS

Plant material

The experiment focused on the evaluation of the seed yield production of the late-season white cabbage cultivar, 'Silviana', which was patented at the Vegetable Research and Development Station Bacau in 2014. 'Silviana' is a robust and crack-resistant cabbage type with superior yield potential. The cabbage head is uniform and ranges from round to elliptical in longitudinal section, with a height ranging from 19-22 cm and a diameter of 22-25 cm. The calculated shape index falls within the range of 0.8-0.95, and the average weight ranges from 1.7-3.3 kg. The leaves exhibit a delicate texture, and the head form in longitudinal section ranges from round to elliptical, with leaves varying in color from raw green to medium intensity green. Furthermore, 'Silviana' has a high vitamin C concentration (44.50 mg per 100 g) and a cellulose level of up to 1.15%, contributing to its nutritional value. 'Silviana' has the potential to achieve high seed yields of 100 to 120 tons ha^{-1}.

Site description

The present investigation was carried out at the experimental field of the Vegetable Research and Development Station Bacau, situated at geographic coordinates 46.585205 N, 26.950087 E, in Romania's north-eastern Bacau region. The study involved a fertile and well-developed soil, characterized by a loamy-sandy texture and polished cambic chernozem soil type. The soil pH was observed to be within the range of 6.2 to 6.7, while the humus content was found to be in the range of 2.5 to 3.5%, making it an ideal soil type for the cultivation of the target crop.

Experimental design

For the experimental investigations, 40-day-old cabbage seedlings of the 'Silviana' cultivar were utilized. The seedling was sown in alveolar palettes in accordance with three distinct time periods and transplanted into the experimental field according to the following periods. The first planting period occurred on July 10th (early), the second on August 10th (mid-season), and the third on September 10th (late). Within each time period, three different planting densities were established. The lowest planting density was achieved using a spacing of 55 x 96 cm (low), resulting in an approximate yield of 18,900 plants ha^{-1}. The medium planting density was obtained with a spacing of 40 x 96 cm (medium), resulting in an average yield of approximately 26,000 plants ha^{-1}. The highest planting density was obtained with a spacing of 25 x 96 cm (high) between plants and rows, resulting in an average yield of approximately 41,600 plants ha^{-1}. The research was conducted on two distinct aspects: the investigation of the planted material and its development, specifically the phenology of the plants, and the analysis of seed germination.

Data collection

The study of the planted material involved the analysis of various morphological characters across all three sets of plants, including number of seeds per silique, seed weight per thousand seeds (TSW), total seed quantity per square meter and per hectare.

Germination

The procedures outlined by the International Seed Testing Association (ISTA) were followed when performing germination tests. For germination of cabbage seeds, a SANYO MLR - 351H germinator was utilized. The optimal temperature for germination was maintained at approximately 20 degrees Celsius, with optimal humidity levels of 80-90% and optimal light exposure of 16 hours per day. The germinator was precisely calibrated to maintain these parameters, providing optimal conditions for cabbage seed germination. Additionally, air circulation was regulated to ensure adequate levels of oxygen for germinating seeds.

Daily observations were conducted during the cabbage seed germination experiment to monitor the process evolution. This allowed for the collection of necessary data to evaluate germination percentage, germination time, germination velocity, as well as other relevant observations.

Germination percentage (G %) represents an approximation of the potential germination of the seed population. The equation to calculate germination percentage is:

$$G(\%) = \frac{\sum_{i=1}^{k} n_i}{N} \times 100$$

The germination percentage was relativized (R %) by the following equation (Fitch et al., 2007):

$$R(\%) = \frac{AP}{HP} \times 100$$

Mean germination time (MGT) is a measure of the rate and time spread of the germination. It indicates time spent to germinate or emerge. Following formula was used to calculate the mean germination time (Ellis and Roberts, 1981):

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

Mean germination rate (MGR) is the reciprocal of the mean germination time as shown below (Ranal et al., 2009):

$$\bar{v} = \frac{1}{\bar{t}}$$

The measure of uncertainty of germination process (U) represents the extent of variability associated with the distribution of the relative frequency of seed germination (Labouriau and Valadares, 1976):

$$U = \sum_{i=1}^{k} f_i \log_2 f_i$$

Synchrony of germination process (Z) evaluates the degree of overlapping among individuals of one population. Synchronization index produces a number if and only if there are two seeds finishing the germination process at the same time. It is calculated using the following formula (Labouriau, 1978):

$$Z = \frac{\sum_{i=1}^{k} C_{n_{i,}2}}{C_{\sum n_1 2}}$$

Coefficient of variation of the germination time (CV_t) is calculated by the following expression (Ranal et al., 2009):

$$CV_t = \frac{S_t}{\bar{t}} \times 100$$

Where: $S_t = \sqrt{\frac{\sum_{i=1}^{k} n_i (t_i - \bar{t})^2}{\sum_{i=1}^{k} n_i - 1}}$

Germination index (GI) is an estimate of the time (in days) it takes a certain germination percentage to occur. Germination index can be calculated by using following expression (AOSA and SCST, 1993):

$$GI = \sum_{i=1}^{k} n_i / t_i$$

Coefficient of velocity of germination (CVG) can be calculated using the following expression (Jones and Sanders, 1987):

$$CVG = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \times 100$$

Time to 50% germination (T_{50}) indicates that how much time was taken for half of the seeds to germinate. (T_{50}) can be calculated using the following expression (Coolbear, Francis, and Grierson, 1984):

$$T_{50} = \frac{t_i + \left(\frac{\sum_{i=1}^{k} n_i}{2} - n_i\right)(t_j - t_i)}{n_j - n_i} \times 100$$

Other germination parameters related to time, such as T_{10}, T_{25}, T_{75}, and T_{90}, can be calculated using the same formula as above, but by substituting $\frac{\sum_{i=1}^{k} n_i}{2}$ with $\frac{\sum_{i=1}^{k} n_i}{10}$, $\frac{\sum_{i=1}^{k} n_i}{4}$, $\frac{3\sum_{i=1}^{k} n_i}{4}$ and $\frac{9\sum_{i=1}^{k} n_i}{4}$.

Mean daily germination percent (MDG) it represents the mean number of seeds germinated per day. This can also be defined as the number of seeds germinating daily relative to the maximum number of germinated seeds. It is calculated using the following expression (Adams and Farrish, 1992):

$$\bar{G} = \frac{GP}{T_n}$$

The peak value (PV) represents the sum of seeds that have germinated at the inflection point on the germination curve, where the rate of germination begins to decline. To calculate the peak value, one must determine the maximum quotient by dividing the cumulative germination values by the corresponding incubation time, as outlined in Adams and Farrish's (1992) methodology.

Germination value is obtained by combining both speed and completeness of germination into a composite score as described by Czabator (1962):

$$GV = \text{MDG} \times \text{PV}$$

Data analysis

The gathered morphological data was evaluated using statistical methods. The obtained analytical data was statistically analysed using the IBM SPSS Statistics software, version 26.0. The ANOVA test was applied to investigate the differences between means, followed by the Tukey's posthoc test. A significant difference was considered at a threshold of $P < 0.05$. The results were presented as means ± standard errors.

RESULTS AND DISCUSSIONS

The quantity of obtained seeds is a crucial aspect in the process of producing autumn white cabbage seeds as it directly affects the efficiency and profitability of the cultivation technology. Insufficient seed quantity can lead to a shortage of raw materials, resulting in additional costs and financial losses. In this experiment, the amount of seeds produced by the studied experimental variants was analyzed. These data allow us to better understand the seed production process in autumn cabbage and identify the corresponding technology and any other factors that may affect this process. The results obtained from the analysis of the quantities of seeds obtained from the experimental variants are presented in Table 1.

Table 1. Results regarding the quantities of seeds from the experimental variants

Date x Density	No. of seeds / silique	TSW	Seeds obtained (grams)	G / m^2	kg ha^{-1}
EH	29.41±0.99 ab	5.05±0.15 b	3219.52±182.84 a	127.72±9.75 a	1277.28±97.53 a
EM	35.5±1.72 a	6.17±0.19 a	2445.9±69.52 b	101.91±2.89 ab	1019.12±28.96 ab
EL	30.58±1.69 ab	6.11±0.09 a	1970.42±314.12 b	82.1±13.09 b	821.01±130.88 b
MH	24.88±2.90 bc	4.76±0.10 b	1095.65±88.72 c	45.65±3.69 c	456.52±36.96 c
MM	25.11±2.07 bc	4.73±0.10 b	970.27±111.62 c	40.43±4.65 c	404.28±46.51 c
ML	24.11±1.90 bc	4.65±0.09 b	1009.56±183.28 c	42.06±7.63 c	420.65±76.36 c
LH	26.44±2.67abc	4.78±0.10 b	811.94±88.16 c	33.83±3.67 c	338.31±36.73 c
LM	19.88±2.14 c	4.71±0.12 b	680.77±86.35 c	28.36±3.59 c	283.65±35.98 c
LL	25±2.51 bc	4.62±0.10 b	420.78±58.71 c	17.53±2.44 c	175.33±24.46 c

The values denote the arithmetic mean ± standard error. Lowercase letters represent the results of the Tukey test for $p < 0.05$ (a - represents the highest value, and ns - non-significant) (EH - Early x High; EM - Early x Medium; EL - Early x Low; MH - Mid-season x High; MM - Mid-season x Medium; ML - Mid-season x Low; LH - Late x High; LM - Late x Medium; LL - Late x Low).

Regarding the number of seeds per silique, a significant variation was observed among the studied experimental variants, with values ranging between the means of 19.88 and 35.5, with the minimum recorded on September 10^{th} (late) combined with a planting distance of 40 x 96 cm (medium) (LM), and the maximum recorded on July 10^{th} combined with a planting distance of 40 x 96 cm (EM). These results suggest that the differences among the studied variants have a significant impact on the number of seeds per silique. In terms of the thousand-seed weight (TSW), the average values ranged from 4.62±0.10 g to 6.17±0.19 g, with a significant variation observed among the experimental variants studied. Concerning the total production expressed in grams, the maximum mean value obtained was 3219.52 grams, recorded by the EH combination. Moreover, a significant variation was observed among the studied variants in terms of both the production per square meter and the yield per hectare. These variations suggest that the differences among the experimental variants have a significant impact on the cabbage seed production.

These results suggest that there is an inverse relationship between plant density and individual plant yield, meaning that as plant density increases, the individual plant yield decreases. However, this effect is compensated for by the larger number of plants in high-density treatments, resulting in a higher total production in these variants compared to those with lower density. This can be observed by comparing the total production, production per square meter, and production per hectare, where the values are higher in high-density treatments.

In conclusion, from the analysis of the presented data, it can be observed that the number of seeds per silique, total production, and production per unit area vary significantly among the studied experimental variants. This suggests that there are significant differences in plant performance depending on the cultivation method used and the establishment period. However, these are not the final results, and the experiment must be repeated to confirm these findings and obtain more precise information about the factors that influence autumn cabbage seed production.

The quality of seeds is a crucial factor that directly affects crop performance and production. High-quality seeds have a higher germination percentage, which results in more plants growing properly. Furthermore, good-quality seeds have a shorter germination time, which can lead to faster and better plant growth. Conversely, low-quality seeds are more susceptible to diseases and pests, which can have a detrimental impact on the crop. Therefore, seed quality evaluation is essential to ensure high and profitable production. The purpose of germination tests is to assess seed quality by analysing the percentage of germination and the time required for it. The Tables 2, 3 and 4 presents the results obtained from the germination study, which was based on several specific indicators that were subjected to statistical interpretation.

Table 2. Results regarding the analysis of seed germination capacity (part 1)

Date x Density	G %	R %	MGT	MGR	U
EH	99.33±0.66 ns	99.33±0.66 ns	1.49±0.23 ns	0.70±0.09 ns	0.83±0.06 ns
EM	100±0 ns	100±0 ns	1.75±0.12 ns	0.57±0.04 ns	1.01±0.27 ns
EL	98.66±0.66 ns	98.66±0.66 ns	1.47±0.24 ns	0.71±0.10 ns	0.78±0.11 ns
MH	97.66±0.66 ns	97.66±0.66 ns	1.67±0.10 ns	0.60±0.04 ns	1.17±0.03 ns
MM	98.66±0.88 ns	98.66±0.88 ns	1.63±0.06 ns	0.61±0.02 ns	1.28±0.12 ns
ML	98.33±0.33 ns	98.33±0.33 ns	1.54±0.09 ns	0.65±0.03 ns	1.24±0.11 ns
LH	98.66±0.66 ns	98.66±0.66 ns	1.61±0.11 ns	0.62±0.04 ns	1.19±0.05 ns
LM	99.33±0.66 ns	99.33±0.66 ns	1.54±0.11 ns	0.65±0.05 ns	1.03±0.08 ns
LL	99.33±0.33 ns	99.33±0.33 ns	1.61±0.19 ns	0.63±0.08 ns	0.92±0.16 ns

The values denote the arithmetic mean ± standard error. Lowercase letters represent the results of the Tukey test for $p < 0.05$ (ns - non-significant) (EH - Early x High; EM - Early x Medium; EL - Early x Low; MH - Mid-season x High; MM - Mid-season x Medium; ML - Mid-season x Low; LH - Late x High; LM - Late x Medium; LL - Late x Low).

Table 3. Results regarding the analysis of seed germination capacity (part 2)

Date x Density	Z	CVt	GI	CVG	T_{50}
EH	0.66±0.02 ns	33.07±7.02 ns	76.33±11.20 ns	70.25±9.69 ns	1.48±0 ns
EM	0.58±0.13 ns	30.48±8.83 ns	64.63±7.03 ns	57.73±4.05 ns	1.36±0.13 ns
EL	0.67±0.08 ns	30.64±4.33 ns	76.22±11.53 ns	71.39±10.88 ns	1.46±0 ns
MH	0.49±0.005 ns	33.88±1.78 ns	66.64±4.32 ns	60.34±4.05 ns	1.32±0.04 ns
MM	0.45±0.03 ns	38.54±3.67 ns	70±2.88 ns	61.35±2.69 ns	1.16±0.10 ns
ML	0.46±0.02 ns	39.65±1.70 ns	73.77±3.80 ns	65.17±3.93 ns	1.22±0 ns
LH	0.47±0.01 ns	35.50±1.58 ns	70.28±4.83 ns	62.66±4.37 ns	1.23±0.11 ns
LM	0.52±0.02 ns	33.84±2.61 ns	73.03±5.01 ns	65.6±5.11 ns	1.18±0.10 ns
LL	0.60±0.07 ns	29.73±4.38 ns	69.83±9.29 ns	63.88±8.42 ns	1.35±0.05 ns

The values denote the arithmetic mean ± standard error. Lowercase letters represent the results of the Tukey test for $p < 0.05$ (ns - non-significant) (EH - Early x High; EM - Early x Medium; EL - Early x Low; MH - Mid-season x High; MM - Mid-season x Medium; ML - Mid-season x Low; LH - Late x High; LM - Late x Medium; LL - Late x Low).

The statistical analysis of the germination capacity of cabbage seeds revealed a low variation between the different variants tested. The percentage of germination (G %) showed a low variation of means, ranging from 97.66 to 100 percent. The lowest germination percentage was recorded by the experimental variant MH, while the highest was recorded by the variant EM. The germination percentage is directly proportional to the number of seeds germinated because 100 seeds were taken under examination for each repetition of the tested variants. The relative germination percentage (R %) displayed the same values as the germination percentage indicator (G %).

The mean germination time (MGT) values ranged from 1.47 days, obtained by variant EL to 1.75 days, obtained by variant EM. The mean germination rate (MGR) is an indicator that can be also expressed as a percentage, showing an average percentage of seed germination of approximately 60-70% for all studied variants, with a variation of about 4-10%. These values are similar and fall within the acceptable limits for cabbage seed germination.

Regarding the uncertainty of the germination process (U), it can be noted that slightly higher variations in the mean were observed, with a minimum of 0.78 recorded by the EL combination and a maximum of 1.28 attained by the MM variant. It should also be noted that a low value of uncertainty (U) indicates a higher degree of uniformity, with a more concentrated and synchronized germination. This can be considered a positive indicator of seed quality, as it suggests better germination capacity and more balanced plant growth.

Regarding the means obtained by the synchrony of germination process (Z), a non-significant variability was observed between the experimental variants, with 0.66 being the maximum value recorded by the variant EH and 0.47 being the lowest value recorded by LH combination. Higher values of this indicator indicate more synchronized germination, and thus it is considered a positive indicator of seed quality.

In the case of the coefficient of variation of germination time (CV_t), a relatively insignificant variation was observed in the means obtained, with the highest mean of 39.65 being attained by the variant ML and the lowest value of 29.73 being recorded by the variant

LL. Regarding the germination index (GI), a non-significant variability was observed among the studied variants, with the highest value of 76.33 being marked by the EH factors combination and the lowest value of 64.63 being attained by the variant EM. A lower value of this indicator illustrates a shorter time to reach the germination percentage, and thus is considered a positive indicator of seed quality.

The coefficient of velocity of germination (CVG) measures the uniformity of germination time for seeds in a sample. The range of CVG values obtained suggests that there is variability in the uniformity of germination times across the different samples tested. The highest value of 71.39 is marked by EL combination, thus indicate that the germination times for the seeds in those samples were less uniform, while the lower CVG values of 57.73 being attained by EM variant. A lower value of this indicator implies a positive future of seed quality.

Table 4. Results regarding the analysis of seed germination capacity (part 3)

Date x Density	T_{90}	MDG	PV	GV
EH	1.75±0.11 ns	7.09±0.04 ns	67±10.01 ns	474.95±69.99 ns
EM	2.14±0.22 ns	7.14±0 ns	50±2 ns	357.14±14.28 ns
EL	1.56±0.27 ns	7.04±0.08 ns	66±12.16 ns	465.04±84.96 ns
MH	1.90±0.04 ns	6.97±0.08 ns	48.5±2.75 ns	338.2±17.99 ns
MM	2±0.13 ns	7.04±0.10 ns	47.83±1.58 ns	337.27±13.82 ns
ML	1.89±0.06 ns	7.02±0.04 ns	53.5±4.64 ns	375.83±32.9 ns
LH	1.89±0.05 ns	7.04±0.08 ns	50.3±4.41 ns	354.59±30.28 ns
LM	1.81±0.06 ns	7.09±0.08 ns	54.5±5.75 ns	386.14±37.94 ns
LL	1.81±0.11 ns	7.09±0.04 ns	56.8±9.63 ns	403.7±69.94 ns

The values denote the arithmetic mean ± standard error. Lowercase letters represent the results of the Tukey test for $p < 0.05$ (ns - non-significant) (EH - Early x High; EM - Early x Medium; EL - Early x Low; MH - Mid-season x High; MM - Mid-season x Medium; ML - Mid-season x Low; LH - Late x High; LM - Late x Medium; LL - Late x Low).

In terms of the time for 50% of the seeds to germinate, the average results varied insignificantly, with a minimum of 1.16 recorded for the variant MM and a maximum of 1.48 recorded for the EH variant. The averages for the time to 90% germination varied between 1.75 and 2.14, with a minimum recorded for EH experimental factors combination and a maximum recorded by EM variant. It needs to be stated that low values of T_{50} and T_{90} indicators express faster germination, thus a low value indicates good germination quality of the seeds subjected to the experiment.

In relation to the mean daily germination percentage (MDG), there was an insignificant variation in the means of the studied variants, with a minimum of 6.97 recorded for the variant MH and a maximum mean of 7.14 recorded for the EM combination.

In the case of the peak value (PV), there was a non-significant variability observed among the examined variants, with a minimum mean of 47.83 marked by the variant MM and a maximum mean of 67 marked by the EH variant.

Regarding the obtained means of the germination value (GV) indicator, a low variability of means can be observed, with a minimum of 337.27 marked by the MM variant and a maximum of 474.95 marked by the variant EH.

These results indicate a high germination rate with low variations among the studied variants. This suggests that the seeds used in the study have good quality and should provide efficient germination when used in agricultural practices.

CONCLUSIONS

The results of the study on the quantities of seeds obtained from experimental variants show significant variations in the number of seeds per silique, total production, and production per unit area. These significant differences suggest that the cultivation density and the time of establishment have a strong impact on plant performance and, consequently, on the quantities of seeds obtained. However, these are provisional observations, and it is necessary to repeat the experiment to confirm these results and better understand the factors influencing seed production in autumn cabbage.

Regarding the seed germination capacity obtained from transplanted mother plants, the results indicate high germination rates, which can be interpreted as an adequate level of seed vigor. Additionally, the low and insignificant variations among the studied variants suggest a uniform production process, which can be an important factor in ensuring high yields in seed production. These results are essential for farmers trying to optimize their production and ensure access to high-quality seeds for their crops.

The study results presented here examine the germination performance of autumn cabbage seeds obtained through various experimental variants and the analysis of key morphological characteristics of generative cabbage plants. This is crucial for producing high-quality seeds and improving yield. The 'Silviana' variety is one of the most important types of autumn cabbage cultivated in Romania, developed through research conducted at the Bacău Research and Development Station for Vegetable Cultivation.

While some of the results obtained cannot be fully explained by the collected data, this highlights the importance of continuing research and investigating these unconventional results to fully understand the mechanisms and processes involved. Further research is necessary to provide a clearer picture of the processes required for optimizing the technology of autumn cabbage seed production. These findings are significant for farmers seeking to maximize yield and obtain high-quality seeds.

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ALLELOPATHIC POTENTIAL ASSESSMENT OF DIFFERENT VEGETAL EXTRACTS ON LETTUCE SEED GERMINATION

Mariana CALARA[1], Neculai MUNTEANU[2], Creola BREZEANU[1], Petre Marian BREZEANU[1], Dan Ioan AVASILOAIEI[1]

[1]Vegetable Research and Development Station Bacau, 220 Calea Barladului Street, Bacau, Romania
[2]University of Life Sciences "Ion Ionescu de la Brad" Iasi, Faculty of Horticulture, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: avasiloaiei_dan_ioan@yahoo.com

Abstract

The aim of the present work was to evaluate the allelopathic influence of different vegetal extracts on the seed germination of lettuce (Lactuca sativa) as test plant. Eight experimental variants have been studied (including Control). The species used for aqueous extracts were: Amaranthus retroflexus, Aquilegia vulgaris, Atriplex hortensis, Chenopodium ficifolium, Cirsium avense, Echinochloa crus-galli, Elytrigia intermedia, Juglans regia, Lactuca serriola, Leucanthemum vulgare, Parthenocissus inserta, Phragmites australis, Poa pratensis, Polygonum aviculare, Portulaca oleracea, Sambuscus ebulus, Solidago gigantea, Xanthium strumarium, Convolvulus arvensis, Vicia sativa, Pinus sylvestris, Helianthus tuberosus. Aqueous extracts from fresh biomass were examined under laboratory bioassay in Petri dishes. The obtained results suggest that the extract from common vetch, canada thistle, red-root amaranth, common purslane and cockspur display more serious allelopathic effects on the seed germination of lettuce. Thus, the study of vegetal extracts with potentials allelopathic effect can lead to development of some new herbicides with low environmental impact.

***Key words**: aqueous extracts, allelopathic effects, new herbicides, environmental impact.*

INTRODUCTION

Allelopathy is a chemical interaction between plants, that could play an important role in natural ecosystems and agro-ecosystems (Molisch, 1937). Various crop species have shown allelopathic activity that can be useful to control weeds in field crops by using them as cover crops, mulch, crop rotation, intercropping, and crop extract (Jabran & Farooq, 2012). Many allelochemicals that can suppress the growth of other plant species have been identified in different weeds species (Siyar et al., 2019). Weed species have allelopathic properties, exactly like crops. Allelochemicals produced by weeds are supposed to be more toxic, because weeds usually grow under stress conditions (Om et al., 2002; Hamburdă et al., 2015). The effects of allelopathic compounds on germination and growth of plants may occur through a variety of mechanisms, including reduced mitotic activity in roots and hypocotyls, reduced rate of ion uptake, suppressed hormone activity, inhibited protein formation, inhibited photosynthesis and respiration, decreased permeability of cell membranes and/or inhibition of enzyme action (Rice, 1974; Corbu et al., 2007). Possibility to use weeds species as an ingredient for the production of herbicides can be an environment-friendly option to control weeds in crops. Herbicides with low environmental impact and new modes of actions are needed due to fast-increasing herbicide resistance in weeds against all the major herbicide groups (Heap, 2018). Weed management in organic production systems is a huge challenge (Munteanu & Stoleru, 2012; Melander et al., 2018). Numerous natural herbicidal substances have been identified from different microbes and crop species (Duke et al., 2000; Jabran, 2017). These herbicidal compounds can be classified in two main groups: terpenoids and phenolics (Anaya, 2006). These natural phytotoxins offer a huge opportunity to be directly used as natural herbicides and to develop new herbicide mode of actions (Dayan & Duke, 2014).

The goal of the present work was to evaluate the allelopathic influence of different species extracts: red-root amaranth (*Amaranthus retroflexus*), common columbine (*Aquilegia* *vulgaris*), orach (*Atriplex hortensis*), fig-leaved goosefoot (*Chenopodium ficifolium*), canada thistle (*Cirsium avense*), cockspur (*Echinochloa crus-galli*), intermediate wheatgrass (*Elytrigia intermedia*), common walnut (*Juglans regia*), prickly lettuce (*Lactuca serriola*), ox-eye daisy (*Leucanthemum vulgare*), thicket creeper (*Parthenocissus inserta*), common reed (*Phragmites australis*), blue grass (*Poa pratensis*), common knotgrass (*Polygonum aviculare*), common purslane (*Portulaca oleracea*), danewort (*Sambuscus ebulus*), giant goldenrod (*Solidago gigantea*), clotbur (*Xanthium strumarium*), field bindweed (*Convolvulus arvensis*), common vetch (*Vicia sativa*), scots pine (*Pinus sylvestris*) and Jerusalem artichoke (*Helianthus tuberosus*).

MATERIALS AND METHODS

Site description

The experiments were carried out at the Vegetable Research and Development Station of Bacau, during 2022 year.

Preparation of water extract

Plant samples for aqueous extracts were collected from SCDL Bacău research field. These samples were cut into small pieces of 1-2 cm. For V1, V2, V3, V4, V5 and V6 variants, the material was inserted into glass jars and the distilled boiled water was added to the samples (Călin, 2005). For V7 variant, fresh sample was grinded through mortar and pestle. Over the ground material was added distilled water and after that the extract was put in the glass jars. The extracts were made from the whole plant (root, stem, leaf and flower) except common walnut and scots pine, from which only leaves were used.

Experimental design

Eight experimental variants have been studied (including Control) (Table 1).

Table 1. Variants used in the experiment

Variants	Allelopathic species
V1	- fig-leaved goosefoot (7.0 g), prickly lettuce (10.0 g), clotbur (10.0 g), common knotgrass (3.0 g), common walnut (5.0 g)
V2	- common reed (8.0 g), danewort (10.0 g), thicket creeper (10 g), common columbine (6.5 g), field bindweed (6.5 g)
V3	- common vetch (4.0 g), canada thistle (10.0 g), red-root amaranth (8.0 g), common purslane (11.5), cockspur (9.0)
V4	- intermediate wheatgrass (5 g), blue grass (3.0 g), Jerusalem artichoke (8.0 g), giant goldenrod (7.0), scots pine (7.0)
V5	- prickly lettuce (4.0 g), clotbur (4.0 g), canada thistle (4.0 g), cockspur (4.0 g), common reed (4 g)
V6	- clotbur (9.0 g), cockspur (3.5 g), prickly lettuce (2.8 g), red-root amaranth (3.5 g), fig-leaved goosefoot (0.63 g)
V7	- common columbine (3.0 g), ox-eye daisy (3.0 g), canada thistle (4.0 g), clotbur (4.0 g), orach (4.0 g), prickly lettuce (4.0 g), common walnut (3.0 g)
VM	- distilled water (Control)

Aqueous extracts from fresh biomass were examined under laboratory bioassay in Petri dishes. Seeds of lettuce were sown on a filter paper in sterilized Petri dishes. The filter papers were moistened with aqueous extracts (7 ml of the extract was pipetted into each Petri-dish). Control was treated similarly with distilled water. There were three replicates of each treatment with 50 seeds per Petri plate. Plates were regularly checked for moisture.

In order to assess extract`s age efficiency, they were applied at two days - in fermentation (A), respectively, 22 days - fermented (B), after preparation, according to Călin (2005).

The research methods used

The parameters used to compare the germination data were:

1. Final Germination Percentage (FGP);
2. Mean Germination Time (MGT);
3. Germination Rate Index (GRI);
4. First Day of Germination (FDG);
5. Last Day of Germination (LDG),

The final germination percentage (FGP %) represents the total number of seedlings at the end of the test, calculated as follows:

FGP (%) = Final no. of seeds germinated in a seed lot × 100.

Mean Germination Time (MGT) was calculated according to the following formula of Orchard (1977):

$$\text{MTG} = \sum f \cdot x / \sum f$$

Where, f is the number of seeds germinated on day x.

Germination Rate Index (GRI) was calculated according to the following formula of Esechie (1994):

$$\text{GRI} = \frac{G1}{1} + \frac{G2}{2} + \cdots + \frac{Gx}{x}$$

Where, G1 represent germination percentage × 100 at the first day after sowing and G2, germination percentage × 100 at the second day after sowing.

FDG = Day on which the first germination event occurred.

LDG = Day on which the last germination event occurred.

The amount of germinated seeds was counted every day, and each lettuce seed was adjudged as germinated if the radicle has emerged (Wang et al., 2016).

Statistical analysis

Data related to germination were analysed statistically with ANOVA. Means were separated via a Tukey's HSD test at $P \leq 0.05$ in IMB SPSS Statistics 20.

RESULTS AND DISCUSSIONS

Germination percentage was analysed from day 2 to day 10 after imbibing, when no further germination occurred. Germination capacity for lettuce is between 65-85% (Stan et al., 2003).

According to ANOVA, results of final germination percentage for variants with aqueous extracts applied at two days after preparation, showed a significant difference ($p<.001$). Tukey post-hoc analysis indicated that the control variant has significantly more sprouted seeds than V1, V2 and V3. For variant V3, germination was completely inhibited (Figure 1).

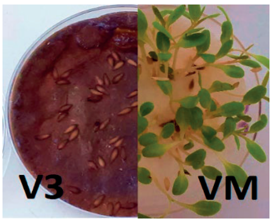

Figure 1. Germination of lettuce seeds, V3 variant and VM - Control variant

Instead, for variant V6, germination was stimulated. Results for aqueous extracts applied at 22 days after preparation highlighted a significant difference in terms of final germination percentage according to ANOVA ($p<.001$). The aqueous extracts exhibit a strong allelopathic activity on lettuce seed germination. Germination was completely inhibited for variants V1, V2, V3 and V4. Based on Tukey post-hoc analysis the control variant has significantly more sprouted seeds than all variants (Figure 2).

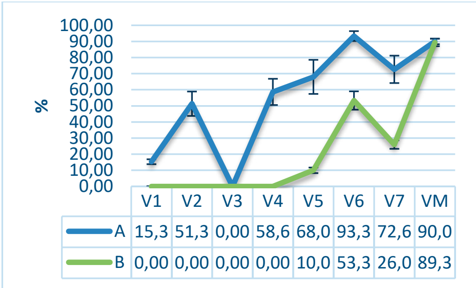

Figure 2. Final Germination Percentage (%): A - Aqueous extracts applied at two days after preparation; B - Aqueous extracts applied at 22 days after preparation

It is interesting that the extract from clotbur, cockspur, prickly lettuce, red-root amaranth and fig-leaved goosefoot have a stimulatory effect on seed germination of lettuce for variant applied at two days after preparation (Figure 3).

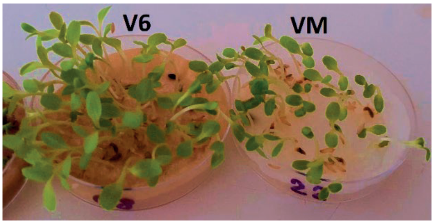

Figure 3. Germination of lettuce seeds, V6 variant and VM - Control variant

Seed germination and rapid germination are generally essential processes in seedling establishment, especially with plants that are endangered. According to Oseni et al. (2020), numerous studies evaluated the germination success only based on final percentage germination, however it is not enough due to the lack of ability to compare two sets of data (one lot of seed may have germinated well before the other, but both achieved the same final germination percentage) and this has led to the development of a number of germination parameters (Jeyavanan et al., 2016). Germination parameter is considered to be a qualitative developmental response of an individual seed that appears at a point in time within a treatment respond within different time

(Murungu, 2011). Initiation of germination is a useful parameter and lower FDG values indicate a faster initiation of germination. ANOVA results revealed a significant influence of the allelopathic extracts on the initiation of germination. The aqueous extracts in V4, V5 V6 applied at two days after preparation, have faster initiation of germination than control. In the case of extracts applied at 22 days after preparation the control variant has a faster initiation of germination (Figure 4).

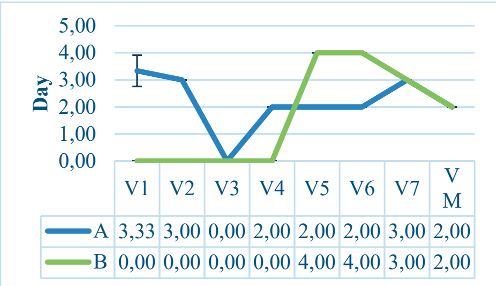

Figure 4. First Day of Germination (FDG) (day): A - Aqueous extracts applied at two days after preparation; B - Aqueous extracts applied at 22 days after preparation

Time at which the last germination event occurred was also considerably different among the treatments. A lower LDG values indicate a faster ending of germination (Kader, 2005). Regarding the LDG for the aqueous extracts applied at two days after preparation, the average value fluctuated between 0.00 and 5.00 days (Figure 5).

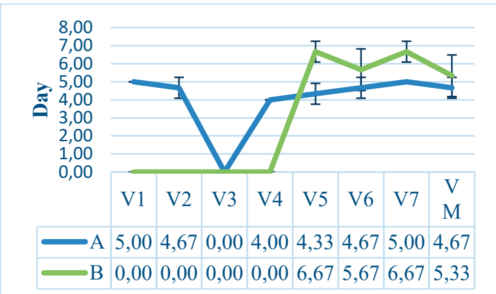

Figure 5. Last Day of Germination (LDG) (day): A - Aqueous extracts applied at two days after preparation; B - Aqueous extracts applied at 22 days after preparation

Value for LDG fluctuated between 0.00 and 0.67 days, with the highest value recorded in V5 and V7, in the case of extracts applied at 22 days after preparation average.

The analysis of variance for germination rate index confirmed that there were significant differences between variants ($p<.001$). Tukey post-hoc analysis indicated that the control variant has significantly a higher GRI than V1, V2, V3, V4 and V7 for extracts applied at two days after preparation. Tukey post-hoc analysis revealed that the control variant has significantly a higher GRI than all variants, for extracts applied at 22 days after preparation (Figure 6). The GRI indicates the percentage of germination on each day of the germination period. Higher GRI values suggest higher and faster germination.

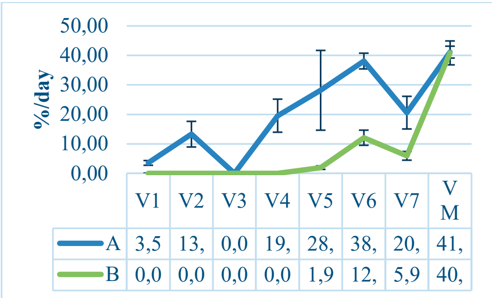

Figure 6. Germination Rate Index (GRI) (%/day): A - Aqueous extracts applied at two days after preparation; B - Aqueous extracts applied at 22 days after preparation

Average value for MTG fluctuated between 0.00 and 4.45, with the highest value recorded in V1, in the case of extracts applied at two days after preparation (Figure 7).

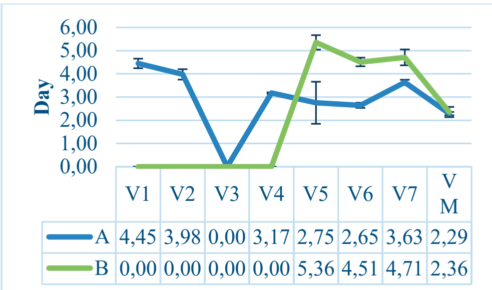

Figure 7. Mean Germination Time (MTG) (day): A - Aqueous extracts applied at two days after preparation; B - Aqueous extracts applied at 22 days after preparation

Instead, extracts applied at 22 days after preparation, average value for MTG fluctuated between 0.00 and 5.36, with the highest value recorded in V5. According to Orchard (1977), the lower the MGT means the faster a population of seeds has germinated.

Further investigation may be necessary to identify the specific compounds in the extracts that led to the inhibition of germination and to determine the potential implications for the practical use of these extracts in agriculture.

CONCLUSIONS

The obtained results suggest that the extract applied at 22 days after preparation display more serious allelopathic effects on the seed germination of lettuce.

The extract from common vetch, canada thistle, red-root amaranth, common purslane and cockspur exhibited a strong allelopathic activity on lettuce seed germination.

The extract from clotbur, cockspur, prickly lettuce, red-root amaranth and fig-leaved goosefoot applied at two days after preparation showed a stimulatory effect on seed germination of lettuce.

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CHANGES OF MORPHOGENETIC PATTERNS OF PLANTS CULTIVATED *IN VITRO* UNDER THE INFLUENCE OF SALICYLIC ACID EMPLOYED AS A TRIGGER OF ANTIOXIDANT DEFENCE MECANISMS IN CABBAGE PLANTS

Tina Oana CRISTEA, Gabriel-Alin IOSOB, Alexandru BUTE

Vegetable Research and Development Station Bacau, 220 Calea Bârladului Street, Bacău, Romania

Corresponding author email: iosob.gabriel@gmail.com

Abstract

Salicylic acid is a stress-signal molecule that is involved in the modulation of growth and development of plants. There is evidence that this stress-signal molecule has a positive impact on the antioxidant defence system, but few studies have been done on determining the most effective concentration and its impact on the growth and development of cabbage plants cultivated in vitro. In the present experiment, we assessed the impact of three different concentrations of salicylic acid (1 mM, 0.5 mM and 0.1 mM) on seed germination indexes, shoot initiation and proliferation, root development, as well as physiologic traits (phenolic and chlorophyll content). It has been found that a concentration of 1mM SA inhibited both seed germination and shoot development, which is probably related with suppression of GA-mediated pathway. Instead, the addition of 0.1 mM SA stimulated shoot proliferation rate, shortened the time for shoot initiation and increased shoot and root elongation. Our results provide the foundation for further studies related to the plant`s agronomic performances when cultivated in the field.

Key words*: shoot, roots, regeneration, defence-related process, germination.*

INTRODUCTION

Salicylic acid is widely distributed plant hormone that has positive impact on a large number of physiological processes such as seed germination, plant signalling and plant response to biotic and abiotic stress. One of the major abiotic stresses is drought, which affects crop growth and yield and thus it is a major constraint for plant productivity worldwide (Lefevere, Bauters, & Gheysen, 2020).

Under the pressure of climatic changes, the effects of drought stress are expected to increase more deepening the water crisis and the availability of water for plants. Drought stress adversely affects a variety of vital physiological and biochemical processes in plants (Hasanuzzaman et al., 2012), causing important loss in plant production.

Thus, nowadays the researches are focusing on deciphering whether exogenously-applied SA can reduce drought stress impact on plants. Up to now, various levels of SA have been shown to protect the buds of *Vitis* genotypes during cryopreservation (Pathirana et al., 2015) and to stimulate flowering in *Eleusine coracana* L. (Appu & Muthukrishnan, 2014) and fruit development in *Fragaria* x *ananassa* Duch. (Kazemi, 2013), *Malus domestica* Borkh. (Shaaban et al., 2011), and *Mangifera indica* L. (Ngullie et al., 2014).

The literature shows that SA, alone or in combination with other compounds (such as jasmonic acid), played the role of elicitor of secondary metabolites in various species such as: *Vitis vinifera* L. (Xu et al., 2015), *Bacopa monnieri* L. (Xu et al., 2015), *Silybum marianum* L. (Ahmed et al., 2020), *Carthamus tinctorius* L. (Golkar et al., 2019), and *Hypericum perforatum* L. (Gadzovska et al., 2013).According to literature, SA affects other physiological processes in plants such as growth, photosynthesis, uptake of ions, heat production, flowering and ethylene production (Ghorbani Javid et al., 2011).At the same time, SA was reported to induce an increase of *in vitro* regeneration frequency in Hibiscus plants (Sakhanokho & Kelley, 2009) and to induce water stress tolerance in Satureja hortensis (Yazdanpanah et al., 2011). However, the efficiency of exogenous SA depends on the species, developmental stage of the plant, the type of application and the concentration of SA (Joseph et al., 2010; Pasternak et al., 2019). The review of literature showed that there are few methods of SA application employed: soaking the seeds prior to sowing, adding to the hydroponic solutions and tissue culture media, irrigating and spraying with SA solution and all revealed the protective role of SA against abiotic and biotic stress agents (Radwan et al., 2008; Sakhabutdinova et al., 2003). The ability of SA to mitigate against hostile effects of salinity in crop plants has been reviewed by Ghorbani Javid et al. (2011).

The plant tissue culture *in vitro* is one of the most effective experimental models in the investigation of various aspects related to the structure and functions of the plant cell and tissues. The formula of basic culture medium provides all nutrients, energy and water necessary for plantlets, organs, tissues or cells growth and the regulation of developmental generally requires the addition of plant growth regulators (PGRs). Successful culture strictly depends on a selection of appropriate PGRs and their concentration and combination with other PGRs. The most commonly used PGRs are auxins and cytokinins. Auxins mediate cell division while cytokinins mediate cell differentiation (Moubayidin et al. 2009). However, cultured plant tissues are also influenced by gibberellins (GAs), brassinosteroids (BRs), abscisic acid (ABA), salicylic acid (SA), jasmonates (JAs) and interactions among them (Gaspar et al., 1996; Phillips and Garda, 2019).

Thus, in our experiment we assessed the impact of three different concentration of salicylic acid (1 mM/l, 0.5 mM/l and 0.1 mM/l) on seed germination indexes, shoot initiation and proliferation, root development as well as physiologic traits (phenolic and chlorophyll content).

MATERIALS AND METHODS

Seeds of *Brassica oleracea,* CT genotype, were used in the experiments developed at Vegetable Research and Development Station Bacau, in The Laboratory of Tissue Culture *in vitro*, aiming to determine the impact of exogenous application of SA.

The biological material was surface sterilised by immersion in mercuric chloride solution ($HgCl_2$) 0.1% for 10 minutes, followed by repeated washing with sterile distilled water.

The sterile seeds were cultivated on Murashige et Skoogmedium (1962) supplemented with 30 g/l sucrose and solidified with 8.0 g/l of agar. The pH was adjusted to 5.8 prior to the addition of the agar.

Three different concentrations of salicylic acid (1 mM, 0.5 mM and 0.1 mM) were added to media and autoclaved at 121°C (1.06 kg/cm^2) for 25 min. Germination indexes were recorded and the one-week-old seedlings were used as source of explants, namely apexes and hypocotyls.

The excised explants were cultivated on the same previously mentioned variants: V0 - control without SA, V1 - 1 mM SA, V2 - 0.5 mM SA, V3 - 0.1 mM SA.

Cultures were then incubated at 26±1°C, a 16-h photoperiod, and 5000 lx light intensity. Repeated sub cultures were done at an interval of 30 days, and day to day observation was carried out to note the responses. The newly formed shoots exhibited the ability to form roots on the same nutritive medium used for shoot micropropagation, which allows the separation of rooted plantlets and the continuation of microclonation process.

The rooted plantlets were transferred to hydroponics conditions in Erlenmeyer flasks 30 mL for acclimatization, while the newly formed shoots were transplanted in new media for their further development. The pots with the hydroponic solution (that contained Previcur - propamocarb clorhidrat 530 g/l + + fosetil de aluminiu 310 g/l in a concentration of 0.15%) were covered with clear bags to provide 100% relative humidity. They were placed in an acclimatization room under a 16/8 h photoperiod at 20-23°C.

The acclimatized plants were planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to green house.

Biometric measurements including plant height, shoot length and root length were recorded 60 days after culture initiation.

Chlorophyll content was determined using Lichtenthaler (1987) protocol. Fresh plant samples (0.1 g) were homogenized and extracted with 80% acetone. The samples were centrifuged for 10 min at 3,530 rpm in the Hettich Universal Centrifuge 320 | 320 R. After collecting the supernatant (SN), the absorbance at 470, 647 and 663 nm was read using UV-VIS spectrophotometer and the concentrations of Cla, Clb and Car were quantified using the following formulas:

$$Cl_a(mg/L) = 12,25 * Abs\ 663\ nm - 2,79 * Abs\ 647\ nm \quad (1)$$

$$Cl_b(mg/L) = 21,50 * Abs\ 647\ nm - 5,10 * Abs\ 663\ nm \quad (2)$$

$$Car\ (mg/L) = \frac{1000 * Abs\ 470\ nm - 1,82 * Cl_a - 85,02 * Cl_b}{198} \quad (3)$$

All variants we tested in three replications and the results were analysed using ANOVA.

RESULTS AND DISCUSSIONS

Under the cultivation techniques tested in our experiment, a concentration of 1 mM SA delayed the seed germination with almost 3 days and the plants exhibited root deficiencies, from lack of roots till very small and abnormal development (Figure 1).

Figure 1. Plants with deficiencies in root development on variants with 1 mM SA

Instead, a concentration of 0.1 mM exhibited similar results with the control variant, the germination rate and shoot growth being positively influenced by the presence in the culture medium of SA (Figure 2). An increased germination percent at low SA concentrations and a decreased percent at higher levels, was reported also in other experiments on carrots, cucumbers, and wheat (Rajasekaran, 2002).
Regarding the morphogenetic reaction of explants, apexes were more suitable for cultivation, new shoots rapidly appeared at the base of the explant and shoot multiplication and elongation was also promoted by the addition of 0.1 mM SA.

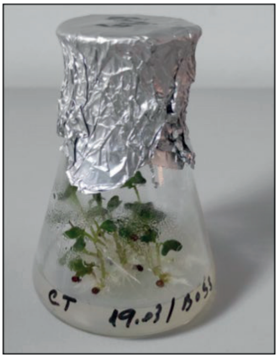


Figure2. Plants with normal root development on variants with 0.1 mM SA

Hypocotyls generated mainly roots and at one bottom of explants, shoots that evolved normally in fully grown plants (Figure 3).
The percentage of shoots producing explants was 93.5% in elicitor-free media and media with reduced concentrations of SA, while significantly lower value for explant response was observed for 1 mM SA (72%).

Figure 3. Morphogenetic reaction of hypocotyls on variants with 0.1 mM SA

On variants with high concentration, salicylic acid suppressed growth of explants and they gradually died (Figure 4). The main effect is exposed by roots that are highly affected by the concentration of SA in culture media. This effect was observed in many crops. For example, in *Arabidopsis*, low SA concentrations are not only effective in increasing root growth, but also decrease K^+ leakage from cells due to acute salt stress, whereas high SA concentrations not only inhibit root growth, but also have no impact on K^+ leakage (Pasternack, 2019).

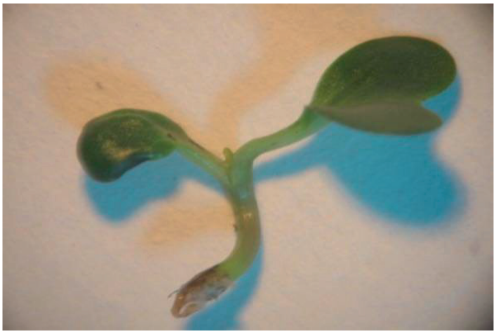


Figure 4. Failure of the plants to develop roots on media with high concentration of SA

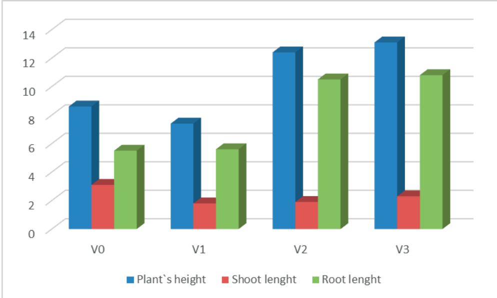


Figure5. Biometric measurements on *Brassica* plants cultivated on media supplemented with different concentration of SA

Our results support the results of Mateo et al., 2006, that concluded that when exogenous SA concentration is higher than 1 mM, plants usually tend to oxidative burst and cell death. Inhibitory effect of high levels of SA has been reported also on somatic embryogenesis in other crops, too. Roust et al. (1989) found that 7 mg l^{-1} SA was optimal for somatic embryo production in carrot suspension cultures, but higher levels (35 mg l^{-1}) were toxic. In 2001, Luo et al. reported that *A. adsurgens* Pall. Plants exhibits a decrease in somatic embryogenesis at levels of SA that are exceeding 24 mg l^{-1}. According to Behzad et al. (2014), a concentration of 2.0 and 5.0 mg l^{-1} of SA, completely inhibited microspore embryogenesis of *Brassica* plants. Although initial divisions were observed, these failed to proceed further into fully developed MDEs. They concluded that the exact toxic level of SA may be genotype-dependent.

SA in concentration of 0.1 and 0.5 mM shortened the time for shoot initiation, increased shoot and root elongation, the results being higher than the control variant (Table 1).

Table 1. Effects of different concentrations of SA in MS medium for multiple shoot induction at *B. oleracea* L. after 60 days of culture - means ±SE

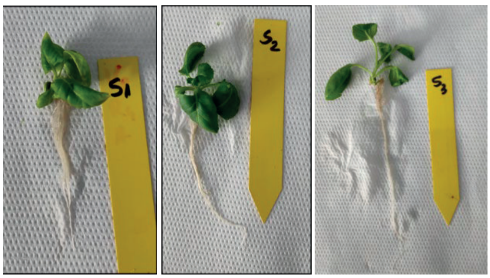
Variant	**% of explant showing response**	**Average no. of shoots**
V0 - Control	95.3	35.6±1.5
V1 - 1 mM SA	72.0	17.0±0.5
V2 – 0.5 mM SA	97.4	44.0±0.5
V3 - 0.1 mM SA	98.8	48.9±2.9

As illustrated in the graphic below (Figure 5), the results obtained proved that 0.1 mM SA promoted the best growth of shoots and roots.

The results indicated highly significant differences in shoot tips performance, demonstrating noticeable effects of SA over organogenesis of cabbage plants grown *in vitro*. A lower concentration of SA promoted both the adventitious shoot formation and their further development in fully grown plants (Figure 6).

Figure 6. Newly formed shoots on variants with 0.1 mM SA

The rooted plantlets (Figure 7) were transferred to the hydroponics conditions and it resulted in more than 95% survival of plantlets.



(a) (b) (c)

Figure7. Rooted plants during first week of hardening: (a) - V1 - 1 mM SA; (b) - V2 - 0.5 mM SA; (c) - V2 - 0.1 mM SA

The total chlorophyll content was enhanced by the addition of the SA elicitor in a concentration of 0.1 mM to the culture media as compared to control. The supplementation of media with SA in higher concentration resulted in similar or a slightly lower Chl a content than in controls (Table 2) d with SA. Our results support other plant studies that had indicated the role of SA in enhancing the photosynthetic pigment content and photosynthesis in different crops (Khan et al., 2003; Khodary, 2004).

Table 2. Effects of different concentrations of SA in MS medium on carotenoids and chlorophyll content of newly formed plants

Variant	Cla (mg/L)	Clb (mg/L)	CAR (mg/L)
V0 - Control	4.49	4.22	0.44
V1 - 1 mM SA	4.12	3.61	0.49
V2 - 0.5 mM SA	4.28	4.24	0.46
V3 - 0.1 mM SA	4.67	4.25	0.43

CONCLUSIONS

The results show that a concentration of 1mM SA inhibited both seed germination and shoot development, which is probably related with suppression of GA-mediated pathway. The markedly negative influence of this concentration of SA on the multiplication rate, shoot formation, and quality of plant tissue registered in our study strengthen the results obtained in other species, where a level of SA above 1 mM is considered high and likely to negatively regulate plant development and growth. The conclusions of our experiment underline the fact that SA highly influences root development starting at seed germination. Also, the cultivation of plants on medium culture with SA often lead to a dwarf phenotype of plants. The shoots are smaller but more robust and the quantity of chlorophyll is increased. This may be explained by a shift of plant response from growth to defence pathways induced by exogenous SA application. Instead, the addition of 0.1 mM SA stimulated shoot proliferation rate, shortened the time for shoot initiation and increased shoot and root elongation.

The content in phenolic and chlorophyll was higher in plants regenerated on this variant also, which indicates the beneficial effect of addition of 0.1mM SA on defence related processes.

Our results provide the foundation for further studies related to the plant`s agronomic performances when cultivated in the field.

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THE EFFECT OF IMPROVING THE CLIMATIC CONDITIONS IN THE GREENHOUSE ON THE CHERAMY TOMATO HYBRID GROWN IN GREENHOUSE CONDITIONS

Ionuț Ovidiu JERCA, Sorin Mihai CÎMPEANU, Răzvan Ionuț TEODORESCU, Jeni ȚIU, Mariana POSTAMENTEL, Adnan ARSHAD, Liliana BĂDULESCU, Elena Maria DRĂGHICI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: draghiciem@yahoo.com

Abstract

Climatic conditions are the most important for the development of tomato culture, namely temperature, humidity and the level of carbon dioxide concentration. The study was carried out in research greenhouses, which belongs to the UASVM in Bucharest on variety Cheramy F1. The environmental factors in the greenhouse, temperature, atmospheric humidity, CO_2 concentration inside the greenhouse. Also, the obtained productions were registered. In this paper we aimed to demonstrate the difference in production of tomatoes obtained in a culture compartment with normal air conditioning equipment and in a compartment in which the climatic conditions were improved by installing heat pump systems.

Key words: *soilless, tomatoes, cherries, Cheramy F1.*

INTRODUCTION

Ground-water heat pumps (also known as geothermal heat pumps) use heat from the ground as a heating source. A technology encouraged and successfully adopted on a large scale in many countries (Sweden, Germany, Finland, France, Switzerland, Italy, etc.) is still in its pioneering phase here. However, more and more people are becoming aware of the real long-term advantages. Ground-water heat pumps have as their operating principle the transfer of heat from the ground to the greenhouse.

The ground water heat pumps help us to keep all climate factors constant throughout the year with a low energy cost and a higher productivity of the tomato culture.

Al Mamun Hossain et al. (2017) conducted studies on leaf area index evaluation using an AccuPAR-LP-80 ceptometer to find out the influence of water amount on tomato leaf size.

One of the major factors affecting the growth and productivity of tomatoes is the very high temperature in the growing space. In general, very high temperatures lead to some physiological disorders but also to a decrease in production.

Hurd and Graves (1984) mention that temperature variations have a negative effect on plant growth, and it has also been reported that it negatively affects nutrient absorption and tomato fruit maturity (Kawasaki et al., 2014; Koskitalo et al., 1972).

Legast et al. (2020) and Falah et al. (2021) in their studies mentioned that the gradual increase in temperature determines the optimization of the phenophase of growth and early development of tomato fruits.

Gruda and Tanny (2015) mentioned that in order to ensure optimal conditions for the best possible fruiting, it is necessary to ensure appropriate day and night temperatures, however, the energy consumption is significant and, therefore, it is necessary to adopt new control approaches of the climate to replace the use of conventional energy, with cogeneration or renewable energy sources, an aspect also emphasized by Li et al. (2015), Shamshiri et al. (2018), Sophoanrith et al. (2021) and Phunchok et al. (2021).

The integrated approach of all technological aspects leads to greater safety of the production obtained (Cañadas et al., 2017)

MATERIALS AND METHODS

The present study was carried out by the Research Greenhouse and the Research Center for Studies of Food and Agricultural Products Quality within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV). The biological material used was the Cheramy RZ F1 hybrid, which is a Dutch hybrid. The tomatoes seeds were procured from Rijk Zwaan with a seed quality certificate. The seedlings were produced in the greenhouse, they were 45 days old when planted. That was planted on 21. X. 2022, on the coconut mats, and 3 plants were planted on each mattress.
The culture was established in two different compartments denoted C12 (C1) and C13 (C2).
All environmental factors in the greenhouse were monitored using the greenhouse computer.
According to the growth and development of the plants, determinations were made regarding the height of the plants, the number of leaves until the first inflorescence, the number of leaves between the inflorescences, the date of the appearance of the first flowers, the date of the appearance of the first set fruits, the date of the appearance of the first fruits at physiological maturity.
Fruit quality determinations were made, fruit mass was determined by weighing each fruit. The content in soluble dry matter was determined by the method of weighing and exposure to the temperature of 105°C for 24 hours, the results being expressed in percent fresh mass (FW). Determinations were made regarding the total production obtained up to the 8th inflorescence. Also, correlations were made regarding the analyzed parameters and vegetation factors, namely temperature, light, carbon dioxide content.

RESULTS AND DISCUSSIONS

After planting the tomato seedlings in the greenhouse, in compartment 12 (C1) we found that average temperature values between 19.8°C and 33.23°C were recorded. Temperatures during the night did not vary much, they were between 15.23°C and 18.55°C. On average, the maximum and minimum temperature values ranged between 25.4°C and 18.72°C (Figure 1).

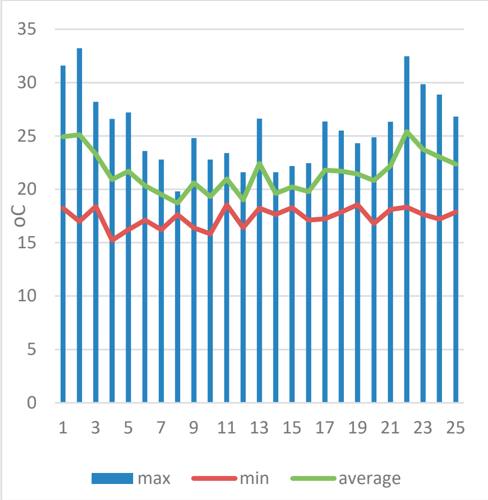


Figure 1. The temperature recorded in the greenhouse during the growth and fruiting period of tomatoes during 25 weeks in compartment 12 (C1)

Analyzing the temperatures outside the greenhouse, it was found that on average the values varied between 11°C and 34°C.
Inside the compartment, the temperature values were kept approximately constant, oscillating between 22.8°C and 24.8°C. The minimum temperatures recorded in this compartment were between 17.5°C and 19.32°C, temperatures corresponding to tomato plants for the night period. Average day-night temperatures between 20.3°C and 21.95 °C were recorded, considered optimal for tomato growth and development (Figure 2).

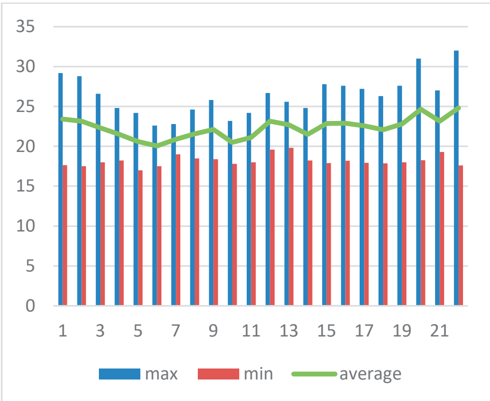


Figure 2. Average temperatures recorded in the greenhouse for 25 weeks after planting tomatoes in the greenhouse in compartment 13 (C2)

Analyzing the variation of the CO_2 content inside the culture space, compartment 12, it was found that the carbon dioxide values varied a lot during the vegetation period. The lowest average value recorded was 157.5 ppm. On average, a value of 252.92 ppm was recorded (Figure 3).

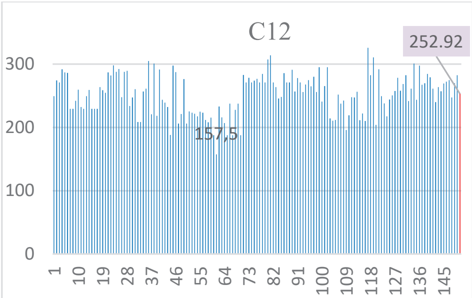


Figure 3. Variation of CO2 content in the greenhouse (C12)

In compartment 13 (C2), the CO_2 concentration remained approximately constant, with an average value of 275.67 ppm. We could appreciate that, since the temperature values remained almost at the programmed level, the ventilation system did not allow the windows to be opened for ventilation, thus, the CO2 content was higher in this compartment, with a beneficial effect on production (Figure 4).

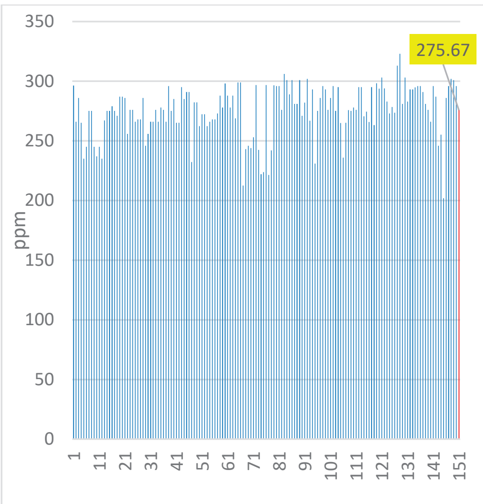


Figure 4. CO_2 content in compartment 13 (C2)

Following the determinations made in March, during the period when the insolation was very strong, weeks 22-23, it was found that in the culture spaces the temperatures varied depending on the position of the compartment. Thus, in C12, a compartment more exposed to solar radiation, the atmospheric temperature varied a lot, an aspect also observed in the culture substrate. Analyzing the temperature values recorded in this compartment, it was found that in the morning at 7:15 the average values were 18.1°C, but at 10:00 the temperature went up to 29.6°C, at 12:00 to 31.4°C, and at 2.30 p.m. at 33.2°C. The temperature recorded in the culture substrate before the application of fertigation, at 10:00 a.m. was 20.7°C, and after the application of fertilization the temperature increased to 21.4 °C in the situation where the temperature of the nutrient solution in the pool was at the value of 25.8°C. At 12:00 the temperature of the substrate was 26.4°C and after fertigation it remained almost constant even though the temperature of the nutrient solution was high, 29.8°C. The highest values were recorded at the substrate level of 30.8°C at 2:30 p.m. After fertigation, the temperature in the substrate recorded high values of 29.9°C, and in the pool the nutrient solution had a temperature of 30.1°C (Figure 5).

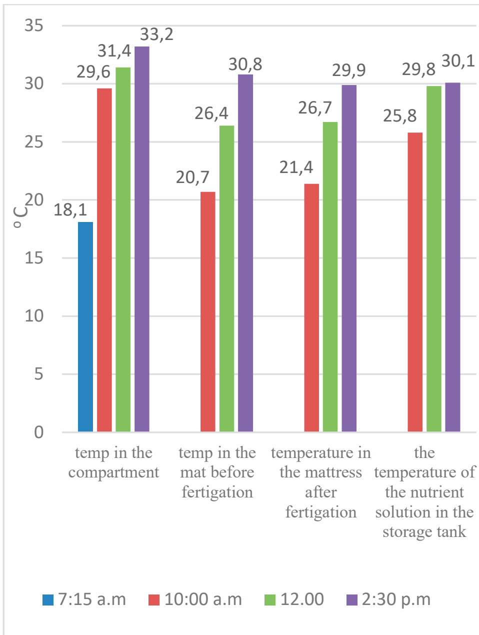


Figure 5. Temperatures recorded in the greenhouse, in the culture substrate before and after fertigation application in compartment C12 (C1)

In compartment 13 (C2) it was found that at 07:15 the average temperature recorded in was also 18.1°C, like that in C12, at 10:00 of 25.8°C, at 12: 00 of 26.3°C, and at 2.30 p.m. of 27.2°C. In this situation, it was found that the temperature values in the culture substrate before fertilizing the temperature remained around 22.7°C, and after fertigation the values remained almost constant at 22.4°C, a situation in which the value the temperature of the nutrient solution in the tank inside the compartment was kept at the approximate value of 22°C. Both at 12:00 and at 2:30 pm the temperatures remained almost constant in the culture substrate, being between 23.6 °C and 23.8°C, and after fertigation the temperatures dropped to 22.7°C and 22.9°C respectively. In the nutrient solution storage pool, the temperature remained almost constant at values between 22.1°C and 22.8°C (Figure 6).

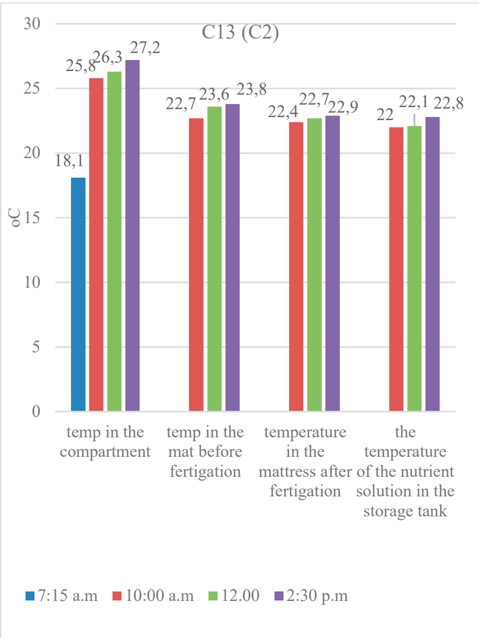


Figure 6. Temperatures recorded in the greenhouse, in the culture substrate before and after the application of fertigation in compartment 13 (C2)

Analyzing by comparison the data on the number of leaves formed until the first inflorescence, it was found that in compartments 12 and 13, the plants formed a number between 7.33 leaves, however, analyzing the average number of leaves between inflorescences, it was found that it was higher (3.66 leaves) in tomato plants grown in compartment 12 compared to compartment 13 where an average of 3.33 leaves were recorded between inflorescences. (Table 1).

Table 1. Number of leaves in tomato plants

Compartment	Number of leaves to first inflorescence	The average number of leaves between the inflorescences
	No.	No.
C12	7.33	3.66
C13	7.33	3.33

Analyzing by comparison the data obtained regarding the distance between the inflorescences, it was found that in the compartment where the temperature values were higher but also oscillating, the distance between the inflorescences was much greater compared to a variant grown in temperature conditions corresponding to the species. In compartment 12 the inflorescences the distance between the inflorescences was smaller, of 24.26 cm, respectively 28.1 cm and due to the fact that the temperatures increased being variable, the distance between the inflorescences was greater, of 34.89 cm, respectively 40.35 cm, values correlated with the very high level of temperature (Figure 7). In the conditions where the temperatures were kept constant and the distance between the inflorescences was almost constant, but much lower compared to that in the compartment where the temperature values varied a lot. Another aspect noted was that the number of fruits in the inflorescences as well as the length of the inflorescence was higher in the case of the compartment where the temperatures were variable.

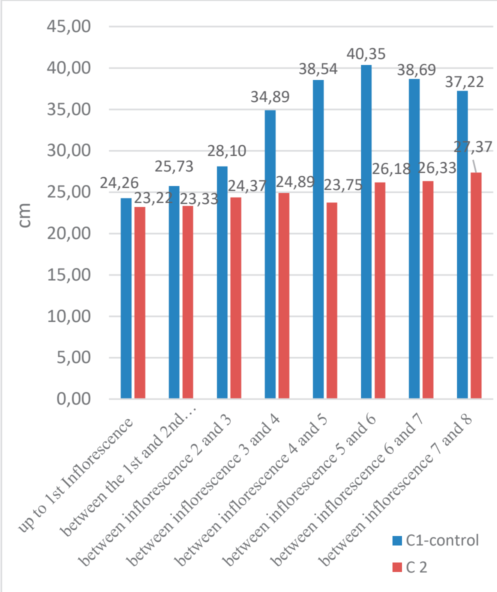


Figure 7. Distance between inflorescences recorded in Cheramy hybrid tomato plants in compartments 12 (C1) and 13 (C2)

Yu and Körner (2020) point out that there is a direct relationship between temperature, CO_2 content and the leaf surface of tomato leaves, an aspect also analyzed by Ouyang et al., (2021) and Quezada (2023) who show that the leaves have a different texture.

The leaf surface was greater by 30,365 cm2 in plants grown in C2 compared to C1, this being 928,5521 cm 2 and 898,1871 cm2 respectively (Figures 8 and 9).

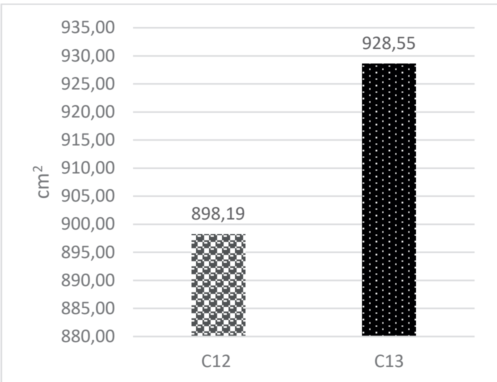


Figure 8. The foliar surface of tomato leaves

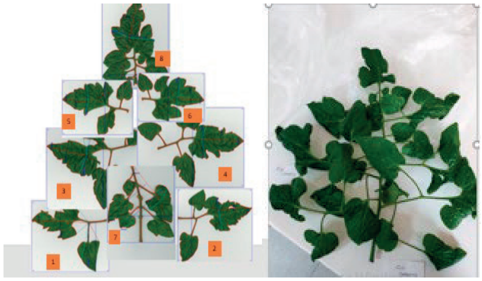


Figure 9. Appearance of leaves harvested from plants grown in C12 and C13 respectively

The dry matter content of tomato leaves was higher in the control variant (C1) of 9.1548% compared to the variant grown in C2 of 7.2864% showing a percentage of only 79.59% compared to the control (Table 2).

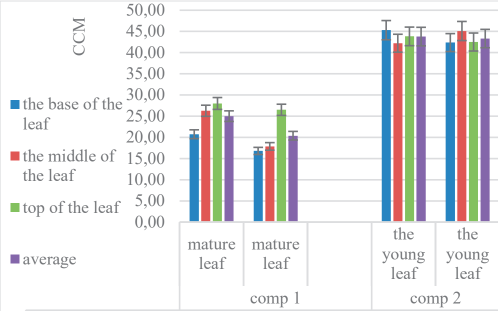
Table 2. Dry matter content of tomato leaves

Compartment	Dry matter content %	Percentage of control
C12 control	9.1548+0.537	100
C13	7.2864+0.316	0.7847

It was noted that the chlorophyll content was different depending on the culture site. On average, following the determinations made at the base of the leaf, in the middle of the leaf and towards the tip of the leaves, the chlorophyll content was higher in compartment 12 (C1) compared to the mature leaves in compartment 13 (C2) being 25.01 CCM, respectively 20.39 CCM. The determinations made on the young leaves showed that, on average, the values were approximately equal to 43.79 and 43.32 CCM, respectively (Figure 10).

Analyzing by comparison the total weight of the fruits obtained per plant, it was found that the average weight of the fruits in the inflorescence was 182.1 g in compartment 12 (C1) and 191.4 g in compartment 13 (C2). From a statistical point of view, there were no significant differences between the 2 compartments regarding the average mass of fruits in the inflorescence.

There were significant differences between the 2 compartments regarding the number of fruits. In compartment 12 (C2) per plant being 17 fruits 1 and 15.4 in compartment 2.



	comp 1 mature leaf	comp 1 mature leaf	comp 2 the young leaf	comp 2 the young leaf
the base of the leaf	20,74	16,80	45,30	42,37
the middle of the leaf	26,28	17,87	42,22	45,10
top of the leaf	28,00	26,50	43,83	42,50
average	25,01	20,39	43,79	43,32

Figure 10. Chlorophyll content of tomato fruits

Regarding the number of fruits formed in the inflorescence, on average, it was higher in plants grown in compartment 12 (C1) 17.0 fruits per inflorescence respectively 15.4 fruits per inflorescence in compartment 13 (C2). From a statistical point of view, distinctly very significant differences were found.

Analyzing the average mass of a fruit, it was found that in compartment 12 (C12) the fruits were of a smaller size of 10.8 g per fruit compared to those in compartment 13 (C2) where the fruits had an average mass of 12.6 g on the fruit. In this case, the differences from a statistical point of view were distinctly very significant both regarding the total number of fruits in inflorescences and the average weight of the fruits (Figure 11).

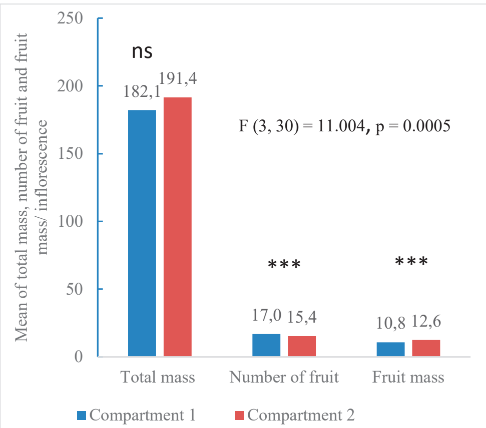
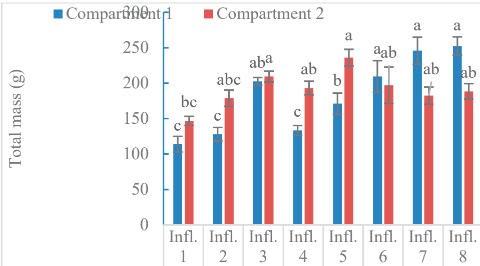


Figure 11. The average mass of fruits in the inflorescence, the number of fruits in the inflorescence and the average mass of a fruit

Analyzing by comparison the total mass of the fruits in the inflorescence, it was possible to find that there were differences between the inflorescences and also between the compartments. Thus, in compartment 12 (C1) at the first inflorescence an average mass of 114 g was determined and in compartment 13 (C2) of 147 g per inflorescence. Starting from inflorescence 6 to inflorescence 8, an increase in the average mass of fruits per total inflorescence was found, this being 209 g at inflorescence 6, 246 g at inflorescence 7 and 252 g at inflorescence 8, an aspect also correlated with the larger number of fruits formed in inflorescences. In compartment 13 (C2) the average mass of fruits per inflorescence did not vary much, being 147 g in the first inflorescence and 236 g in the 5th inflorescence (Figure 12).



	Infl. 1	Infl. 2	Infl. 3	Infl. 4	Infl. 5	Infl. 6	Infl. 7	Infl. 8
Compartment 1	114	128	202	133	171	209	246	252
Compartment 2	147	179	209	193	236	197	182	188

Figure 12. The average mass of fruits from inflorescences 1-8 determined in experimental compartments

The number of fruits in the inflorescence differed according to the position of the inflorescence. It was found that in the case of both cultivation variants in the first 2 inflorescences, the number of fruits was lower compared to the rest of the inflorescences, however, there were differences between the two compartments. In compartment 13 (C2) the number of fruits in the inflorescence was higher compared to those in compartment 12 (C1). In inflorescences 7 and 8, between 25 and 24 fruits were formed per inflorescence, but in compartment 13 (C2) the number of fruits remained almost constant (Figure 13).

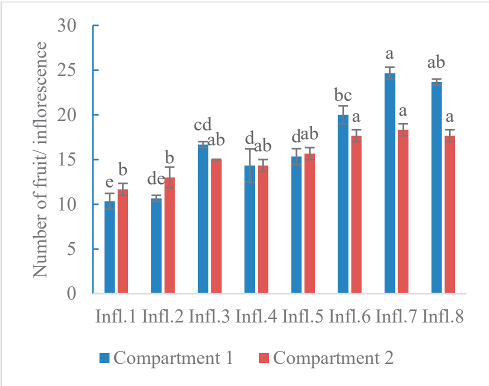


Figure 13. The number of fruits per inflorescence

Analyzing by comparison the average mass of the fruits formed per inflorescence, it was found that in the case of florescence 1 the fruits had an average mass of 11.01 g per fruit for those from compartment 12 (C1) and 12.6 g/fruit for those from compartment 13 (C2). In the case of the rest of the fruits from the inflorescences, it was found that in compartment 13 (C2) the weight of the fruits was higher compared to the weight of the fruits in compartment 12 (C1) (Figure 14).

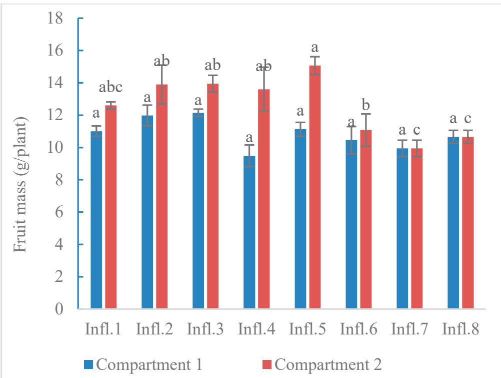


Figure 14. Average mass of a fruit per inflorescence per total plant

The cultural aspects are presented in Figure 15.

C12 C13

Figure 15. Appearance of inflorescences in C1 and C2

CONCLUSIONS

The temperature in the culture space is also influenced by the surface exposed to solar radiation. The distance between the inflorescences was influenced by the temperatures in the culture space. This was higher in tomato plants grown in C12 where temperature values were higher and variable.

The dry matter content of tomato leaves in C1 was higher compared to C2.

On average, per plant, the mass of fruits per inflorescence was between 182.1 g in C1 and 191.4 in C2, the differences being statistically insignificant. But there were significant differences regarding the number of fruits in the inflorescence and the average weight of the fruits.

ACKNOWLEDGEMENTS

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INFLUENCE OF ESSENTIAL OIL SPEARMINT (*MENTHA SPICATA* L.) CULTURE ON SOIL BIOGENICITY AND DETERMINATION OF ITS ANTIMICROBIAL ACTIVITY AGAINST *ESCHERICHIA COLI*

Boyka MALCHEVA[1], Pavlina NASKOVA[2], Dragomir PLAMENOV[2], Dimitar NIKOLOV[3]

[1]University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria
[2]Technical University - Varna, 1 Studentska Str., Sofia, Bulgaria
[3]General Toshevo Municipality - chief architect , 5 V. April Str., General Toshevo, Bulgaria

Corresponding author email: boika.malcheva@gmail.com

Abstract

Soil microbiological and agrochemical indicators were analyzed during biological cultivation of spearmint, in greenhouse conditions, as main indicators of good plant development, studied for antimicrobial activity against Escherichia coli, by testing different variants of plant extracts (decoction, tincture, medicinal wine, medicinal vinegar, medicinal oil). The results of the agrochemical analysis show that spearmint does not have a major impact on the dynamics of macronutrients in the soil. While the biogenicity and activity of enzymes cellulase and catalase increased in the soils with spearmint culture compared to the no-vegetation control. Positive antimicrobial activity against the pathogenic microorganism Escherichia coli was reported for all variants of extracts of spearmint, differing for individual parts of the plant and individual variants of extracts. Root and whole plant extracts showed higher antimicrobial activity compared to leaf and stem extracts. The strongest antimicrobial activity of the plant extracts was found in the medicated oil and medicated vinegar variants and the weakest in the `decoction` variants. The choice of solvent and exposure time likely influence the diameter of the sterile zone.

***Key words**: spearmint, soil microorganisms, cellulase, catalase, antimicrobial activity.*

INTRODUCTION

One of the sources of antimicrobial agents of natural origin can be traditional plants, which contain a whole range of pharmacologically active compounds with pronounced antimicrobial action. Currently, these compounds are of particular interest. Plants accumulate in their tissues an arsenal of protective substances necessary for survival in an extreme environment and in an aggressive neighborhood with pathogenic microorganisms. In this regard, the study of the antimicrobial potential of plants is extremely important for humans. Some plant metabolites, such as flavonoids, alkaloids and terpenes, have pronounced antimicrobial activity.

Plant oils and extracts have been used for a wide variety of purposes for many years. Recently, they have attracted widespread interest as a source of natural antimicrobial compounds. Essential oils and plant extracts are of particular interest because they are relatively safe and have well-pronounced antimicrobial properties. Traditionally, people have used crude extracts from various plant parts as medicinal agents. Plant extracts have also been used to treat infectious diseases caused by antibiotic-resistant microorganisms. In fact, herbal medicines have received much attention as sources of beneficial compounds because they are considered to have stood the test of time, and are relatively safe for human use and environmentally friendly. They are also economical and easily available. Last but not least, natural products are of great chemical diversity and could provide an opportunity to create new drugs. They can be used as a source of pure compounds or as standardized plant extracts. All or any part of the plant, such as bark, leaves, roots, seeds and stems, can be incorporated into the creation of a new pharmaceutical product, as most possess antimicrobial properties. Essential oils are produced from various plant parts (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) as secondary metabolites (Palazzolo et al., 2013). Mentha essential oil is mainly produced from the leaves of the plant (Hiruma, 1993; Monte et al., 2001; Gobert et al., 2002; Lorenzo et al., 2002; Marchese et al., 2005; Sartoratto et al., 2004; Bertini et al., 2005; Bieski, 2005; Pľuchtová et al., 2018).

Mentha spicata L. is a well-known herb of the Lamiaceae family, a rich source of essential oils that are widely used in the pharmaceutical industry, cosmetics, and as a spice in food preparation. It is distributed mainly in the temperate and sub-temperate zones of the world. It is widely cultivated for commercial essential oil production (İcan et al., 2002; Pandey et al., 2003; Lawrence, 2007). *M. spicata* has also gained increased scientific interest, primarily as an antimicrobial and antioxidant bioactive natural extract (Mata et al., 2007). *M. spicata* essential oil demonstrated moderate antioxidant activities as well as moderate to weak antimicrobial activities with the best sensitivity observed for Gram-positive bacteria due to its chemical composition of 44 unique oxidized monoterpene compounds (67.2%), followed by monoterpene hydrocarbons (20.8%) (Bardaweel et al., 2018). The main constituents of *M. spicata* essential oil are phenolic compounds such as carvone and limonene (Telci et al., 2010). According to Bardaweel et al. (2018) spearmint oil exhibited the strongest antibacterial activity against *Staphylococcus epidermis* and *Escherichia coli* and weaker antifungal activity against *Candida glabrata*. *M. spicata* essential oil showed a moderate level of antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*, with *Listeria monocytogenes* being the most sensitive species (Shahbazi, 2015). Oxidized monoterpenes have apparently been reported as a potent antimicrobial agent in the composition of several essential oils (Knobloch et al., 1989). Furthermore, 1,8-cineole and sesquiterpenes have been shown to exhibit significant antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria (Knobloch et al., 1989; Baratta et al., 1998). However, since essential oils contain multiple components, their antimicrobial activity is rather due to additive, synergistic or antagonistic effects of the individual components (Bardaweel et al., 2018).

Essential oils of *Mentha piperita*, *Mentha spicata* var. *crispa* and *Mentha arvensis*, have great antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacillus subtilis* (Horváth and Koščová, 2017; Bokhari et al., 2016; Ullah et al., 2012). *Mentha pulegium* shows activity against *Staphylococcus aureus* and *Enterococcus faecalis* (Aycan et al., 2015). In general, Gram-positive bacteria are more sensitive to the effects of essential oils than Gram-negative bacteria, due to significant structural differences in the cell wall of these two groups of bacteria (Horváth and Koščová, 2017; Cunha et al., 2018; Mancuso et al., 2019). This fact increases the interest in researching the antibacterial activity of rhododendron against Gram-negative bacteria. *Mentha spicata* and other *Mentha* species show activity against Gram-negative bacteria. *Mentha spicata* essential oil has significant antibacterial potential against biofilm cultures of *Vibrio* spp. (Snoussi et al., 2015). *Mentha longifolia* is active against *Salmonella typhimurium* (Heydari et al., 2015). *Mentha pulegium* inhibits the growth of *Pseudomonas* sp., *Escherichia coli* and *Pseudomonas aeruginosa* (Aycan et al., 2015; Moghtader et al., 2016; Sariri et al., 2011). When studying the antibacterial activity of essential oils from *Mentha piperita* and *Mentha spicata* against the species *Enterobacter cloacae*, *Salmonella* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, the same antibacterial activity against Gram-negative bacteria and varying antibacterial activity against Grm was found - positive bacteria (Pľuchtová et al., 2018). *Mentha spicata* essential oil showed good antimicrobial activity against Staphylococcus aureus and *Escherichia coli* due to the high concentration of carvone (67%) (Scherer et al., 2013).

The use of *Mentha* oils and essential oils in general as natural substances and therefore easily biodegradable may be a promising alternative to synthetic materials to combat increasingly common bacterial infections (Mancuso, 2020). The purpose of the present study is, on the one hand, to determine the influence of the essential oil culture of spearmint on the soil microflora, and on the other hand, to analyze the antimicrobial activity of the plant against the pathogenic bacterial species *Escherichia coli*.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse conditions of the educational and experimental field of the Department of Plant Breeding at the Technical University - Varna.

Before planting the experiment, during the growing season and after harvesting the crop, soil samples were taken, for each of the options, to determine:

- ammonium nitrogen (NH_4–N) and nitrate nitrogen (NO_3–N) - photometric method;
- assimilable forms of phosphorus and potassium - Egner-Riem double-lactate method;
- Soil pH (water extract) - ISO 10390.

For the microbiological analysis, the method of dilution and triplicate inoculation of solid nutrient media was used with subsequent counting of colony-forming units (CFU) in 1 g abs. dry soil (Mishustin and Emtsev, 1989; Malcheva and Naskova, 2018; Nustorova and Malcheva, 2020). Systematic and physiological groups of aerobic microbes - bacilli and non-spore-forming bacteria (on ordinary agar), micromycetes (mold fungi) - on Chapek-Dox agar, actinomycetes and bacteria assimilating mineral nitrogen (on Actinomycetes isolation agar) were determined. The general microflora was determined. The mineralization coefficient was calculated according to the formula: bacteria assimilating mineral nitrogen/(non-spore-forming bacteria+bacilli) (Mishustin and Runov, 1957; Malcheva and Naskova, 2018).

To isolate *Escherichia coli*, a solid culture was made on Endo agar. Typical *E. coli* colonies on Endo agar are dark red with a metallic sheen (Malcheva and Naskova, 2020). Certified reference material was used: *Escherichia coli* WDCM 0090 VT000904.

The agar diffusion method was used to determine antimicrobial activity (Nustorova and Malcheva, 2020). The volume of inoculated extract in each well was 60 µl.

The catalase activity of soil microorganisms was determined by the manganometric method (Khaziev, 1976).

In the laboratory experiment to determine the cellulose-decomposing activity, soil with a thickness of about 7 mm was poured into a Petri dish with a diameter of 10 cm, maintaining 60% PPV/maximum field moisture content/. In each Petri dish, 3 strips of sterile filter paper measuring 10/50 mm are placed on the soil and cultivated at 25°C. During 10 days, the area of the degraded cellulose is recorded with a standard mesh. Average values from the three bands are calculated.

The following variants of spearmint extracts were prepared (Table 1).

Table 1. Extract variants

Variant	Method of preparation of the extract	Parts of the plant used
Decoct	A decoction (potion) is the liquid obtained by boiling the chopped plant product with the necessary solvent, usually water. Recommended for roots, flowers, leaves, twigs, fruits. The extractive solution is filtered while hot.	roots leaves stems whole plant
Tincture	Therapeutic substances are extracted from the chopped herbs by soaking with ethyl alcohol at a concentration of 30%, usually for a time varying between 8-10 days. The operation is carried out in well-closed glass containers. Shaking, for good extraction, is necessary throughout the extraction period.	roots leaves stems whole plant
Medicinal wine	The extraction is carried out in a weak hydroalcoholic environment, at a slightly acidic pH. For preparation, pre-crushed herbs are soaked for 7-10 days in wine (of good quality and well stabilized), after which the preparation is filtered.	roots leaves stems whole plant
Medicinal vinegar	It is obtained by extracting the active substances from herbal drugs with wine vinegar. For preparation, previously crushed herbs are soaked for 7-10 days in vinegar (good quality), after which the preparation is filtered.	roots leaves stems whole plant
Medicinal oil	It is a form of soaking the herbs in olive oil. The duration of soaking is 4-6 weeks. Store in tightly closed glass bottles, in a dark and cool place.	roots leaves stems whole plant

RESULTS AND DISCUSSIONS

An agrochemical analysis of soil samples was made before the sowing of spearmint and at the end of the vegetation of the plant species (Table 2).

Table 2. Agrochemical analysis

Variant	pH	Macronutrients			
		NH_4 mg/kg	NO_3 mg/kg	P_2O_5 mg/100 g	K_2O mg/100 g
Control (no vegetation)	7.26	4.03	8.24	17.1	17.7
Mentha spicata L.	7.21	4.01	5.04	14.0	15.1

After the sowing of spearmint, the values of the digestible forms of N, P and K slightly decrease, but comparing with the limit values for stocking of the soil with available nitrogen compounds, mobile phosphates and digestible potassium, it can be concluded that the soil is poorly stocked with nitrogen, but it has a good degree of storage in terms of phosphorus and potassium, and the soil reaction is slightly alkaline, relatively favorable for the development of gorse.

The biogenicity and individual groups of microorganisms (bacteria, actinomycetes, micromycetes) of the studied variants are presented in Table 3.

Table 3. Quantity and qualitative composition of soil microorganisms (CFU x 103/g abs. dry soil)

Variant	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria assimilating mineral nitrogen	Minera-lization coefficient
Control (before placing the trial, no vegetation)	3259.2	2667.2	400	57.6	134.4	4920	1.60
Mentha sativa L. (at the end of the growing season)	3932.8	2860	452.4	282	338.4	5460	1.65

The results show that the biogenicity of the soils is higher in the variant with vegetation compared to the control (no vegetation). This trend applies to the individual studied groups of microorganisms and, accordingly, to the general microflora. The rate of decomposition of organic matter in soils correlates with the amount of microorganisms.

In both variants, non-spore-forming bacteria take the main share in the composition of the general microflora, followed by bacilli, and the least represented are micromycetes (mold fungi) and actinomycetes (Figures 1 and 2).

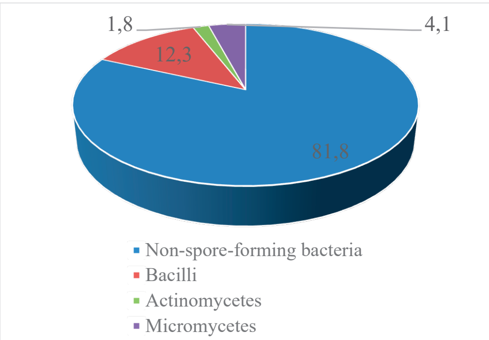

Figure 1. Percentage participation of microorganisms in the composition of the total microflora (control)

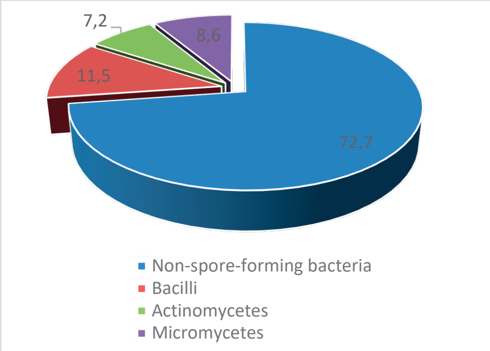

Figure 2. Percentage participation of microorganisms in the composition of the total microflora (*Mentha spicata* L.)

The percentage of non-spore-forming bacteria in the non-vegetated variant is higher, while in the variant with spearmint the amount of this group of microorganisms decreases at the expense of an increase in the amount of actinomycetes and micromycetes. The amount of spore-forming bacteria - bacilli - remains relatively constant in both variants. Non-spore-forming bacteria and bacilli are mainly involved in the initial stages, and actinomycetes and micromycetes in the final stages of decomposition of organic matter.

The catalase activity of the tested variants is presented in Figure 3.

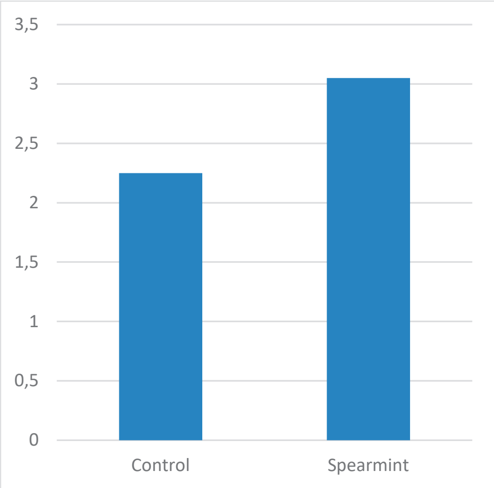

Figure 3. Catalase activity of soil microorganisms ($ml\ O_2/30\ min$)

Catalase is a respiratory enzyme that breaks down the toxic hydrogen peroxide that is released when proteins are broken down. The results show that the catalase activity increases 1.4 times in the variant with sedum compared to the variant without vegetation. Catalase values correlate with the amount of soil microorganisms. There is also catalase of plant origin.

The cellulase activity of the tested variants is presented in Figure 4.

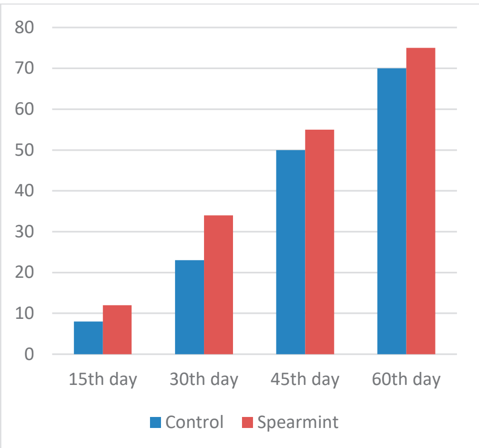

Figure 4. Cellulase activity of soil microorganisms (% degraded area)

Cellulase catalyzes the hydrolysis of cellulose, in which cellulose is initially broken down to cellobiose, which under the action of ß-glucosidase is broken down to glucose. The cellulase activity of the soil microorganisms was followed dynamically for a period of two months under laboratory conditions. The results showed higher cellulase enzyme values in the spearmint variant compared to the control. This trend correlates with the amount, composition and mineralization activity of soil microorganisms.

A number of factors are important for enzyme activity: soil type, soil humidity and temperature, content of nutritional elements, amount and composition of microflora, type of vegetation and others. Microbiological and enzymatic activity are proposed by a number of authors as sensitive soil indicators, including contamination with pathogenic microorganisms (Malcheva et al., 2021; Malcheva et al., 2022, Dilly et al., 2003; Nannipieri et al., 2000; Li et al., 2008; Perucci, 1992; Pascual et al., 1999; García-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004; Bastida et al., 2008; Marcote et al. al., 2001; Malcheva, 2014a, b).

The results were negative for the presence of the tested pathogenic species Escherichia coli in both variants, which allowed to test for antimicrobial activity the prepared variants of plant extracts from roots, leaves, stems and whole plant of spearmint (Table 4). Essential oils are produced from various plant parts (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) as secondary metabolites (Palazzolo et al., 2013). Mentha essential oil is mainly produced from the leaves of the plant (Hiruma, 1993; Monte et al., 2001; Gobert et al., 2002; Lorenzo et al., 2002; Marchese et al., 2005; Sartoratto et al., 2004; Bertini et al., 2005; Bieski, 2005; Pľuchtová et al., 2018).

Table 4. Antimicrobial activity against *Escherichia coli* of the studied extracts of spearmint

Variant	Sterile area, cm			
	Roots	Leaves	Stems	Whole plant
Decoct	0.9	0.2	0.2	0.2
Tincture	0.7	0.5	0.4	0.6
Medicinal wine	1.0	0.5	0.8	1.0
Medicinal vinegar	0.5	0.4	0.4	0.5
Medicinal oil	1.1	0.8	1.0	1.1

The sterile zone for extracts of *Mentha spicata* root decreases in the following order: medicinal oil > medicinal vinegar > decoction > tincture > medicinal wine. Compared to the root extracts, the sterile zone of the leaf extracts had lower values, indicating that the rhododendron roots had a more effective effect against *Escherichia coli* compared to their leaves. The sterile zone for spearmint leaf extracts decreases in the following order: medicinal oil > medicinal vinegar = tincture > medicinal wine > decoction. Compared with the root and leaf extracts, the sterile zone of the stem extracts was intermediate between the root and leaf results of the tincture, medicinal vinegar and medicinal oil, indicating that the coriander roots had a more effective effect and the leaves with weaker effect against *Escherichia coli* compared to its stems. In medicinal wine, the same effect against the pathogen was found for leaf and stem extracts. The sterile zone for coriander stem extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture = medicinal wine > decoction. Compared to the rest of the whole plant extracts, the sterile zone is comparable to root extracts - the largest retention zone compared to leaf and stem extracts (except for decoction). It was found that the sterile zone in the decoction variant was greater only in the root extracts, in the leaf, stem and whole plant extracts it was the same and the lowest. The sterile zone for coriander whole plant extracts decreases in the following order: medicinal oil > medicinal vinegar > tincture > medicinal wine > decoction. In terms of antimicrobial activity, gram-negative bacteria (Escherichia coli and others) are more sensitive to coriander oil than gram-positive bacteria (Saygi et al., 2021; Silva et al., 2011). Similar results against *Escherichia coli* were obtained in the study of antibacterial activity of savory oil (Blažeković et al., 2010). A study of the same variant extracts, but from coriander, found higher soil biogenicity and antibacterial activity against E. coli in coriander compared to coriander (Naskova et al., 2023). Joden oil exhibits moderate (Shahbazi, 2015) and strong (Bardaweel et al., 2018)) antibacterial activity against *Escherichia coli*. The antimicrobial activity of spearmint oil is due to its chemical composition - oxidized monoterpenes, monoterpene hydrocarbons, phenolic compounds such as carvone and limonene (Bardaweel et al., 2018; Telci et al., 2010).

The additional conditions and factors - reducing the pH of the medium when using medicinal vinegar and medicinal wine, the inclusion of additional plants - grapes (wine), vinegar (apples, grapes), olives (solvent olive oil for the medicinal oil), the nature of the solvent (Kačániová et al., 2020) also influence the retention zone. In the variants with medicinal vinegar, a general sterile zone is formed around the wells, in the variants with medicinal wine - a bubble halo at a distance of 0.5 cm around each well - probably reactions from the created acidic environment. In the medicated oil variants, the sterile zone increases towards the interior, showing an enhanced effect of combining the root, leaf, stem, whole plant variants and combining the spearmint extracts with olive extract (olive oil). As the time of action of the extracts increases (24 h, 48 h, 72 h), the diameter of the sterile zone increases by about 0.1 cm. Essential oil from the plant *Satureja hortensis* L. manifested varied antibacterial activity against *Escherichia coli*, *Salmonella enteritidis*, and *Bacillus subtilis*, depending on the concentration of essential oil used as well as the type of bacteria (Blažeković et al., 2010).

Some of the options and results are presented in Figure 5.

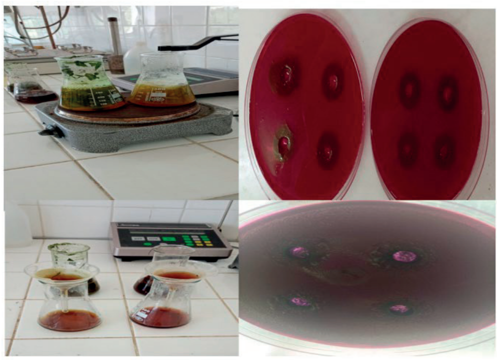

Figure 5. Photo material of some variants and results

CONCLUSIONS

The content of nitrogen, phosphorus and potassium slightly decreases in the variant with spearmint compared to the control, without vegetation, i.e. *Mentha spicata* does not have a major impact on the dynamics of macronutrients in the soil. The macronutrient values obtained were close at the beginning and end of the experiment.

The variants with spearmint increase the biogenicity of soil microorganisms, but in general the composition and percentage participation of the investigated groups of microorganisms is preserved. In all variants, the main share in the composition of the general microflora is occupied by non-spore-forming bacteria, followed by bacilli, and the least represented are actinomycetes and micromycetes.

The values of the enzymes catalase and cellulase correlate with the amount of microorganisms. Their activity is higher in the variant with spearmint compared to the variant without vegetation. A number of factors are important for enzyme activity: soil type, soil humidity and temperature, content of nutritional elements, amount and composition of microflora, type of vegetation and others.

Root and whole plant extracts showed higher antimicrobial activity against *Escherichia coli* compared to leaf and stem extracts. The strongest antimicrobial activity of the plant extracts was found in the medicated oil and medicated vinegar variants and the weakest in the "decoction" variants (except for the root extracts).

A possible reduction in the pH of the medium when using medicinal vinegar and medicinal wine, the inclusion of additional plants, the nature of the solvent, the concentration of the extract and its exposure time also affect the diameter of the retention zone.

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STUDY ON THE INFLUENCE OF NUTRIENT CONDITIONS ON THE PRODUCTION AND QUALITY OF LETTUCE GROWN ON PERLIT SUBSTRATE

Daniela MICU (BALINT), Sovorn CHAN, Elena Maria DRĂGHICI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: draghiciem@yahoo.com

Abstract

The study was carried out in the research greenhouse, within the Horticultural Products Quality Research Center in 2022. A variety of oak leaf lettuce, Kineta, was grown on the mattresses filled with perlite. The nutrient solution was administered in 3 concentrations (EC), namely 1.5 mS/cm^2, 2.5 mS/cm^2 and 3.5 mS/cm^2. Three pH levels were used for each EC type. Differences were found between the experimental variants regarding the variety's reaction to these treatments. The aim of the study was to see the influence of the nutrient solution concentration on some production and quality parameters of lettuce grown on perlite substrate.

***Key words**: lettuce; soilless; perlite; substrate.*

INTRODUCTION

Lactuca sativa L. is one of the vegetable species cultivated on large areas, in all cropping systems, being consumed all year round. The salad assortment is quite varied from head salad to leaf salad. Within the assortment, the color varieties are diverse, from light green to dark red. In greenhouse conditions various cultivation methods have been adopted from soil cultivation practiced especially in solar conditions, spring or autumn sometimes and winter. Since the time to obtain the edible part is longer under the conditions of soil cultivation and the fact that the humidity of the soil cannot be controlled permanently, but also due to the pests that may appear in the soil in the last period, I resorted to the application of different methods of cultivating lettuce in an unconventional system. A frequently encountered method is the cultivation of lettuce on different types of substrates or under culture conditions in a nutrient film technology system. These methods lead to better control of the culture substrate, better monitoring of the nutrient solution, and finally to obtaining a well-controlled product from the point of view of the nitrate content. Different researchers have applied the cultivation system to substrates with very good results, thus being able to recommend the best variants in terms of nutrient solution concentration (Al-Kinani & Draghici, 2009). Simultaneously with the application of the non-conventional system on the culture substrate, organic fertilizers can also be applied, fertilizers that have a positive effect on the chemical composition of lettuce plants (Drăghici et al., 2016; Nicola et al., 2020) but also on the growth of lettuce plants (Enache et al., 2019; Zienab et al., 2021).

Nerlich & Dannehl (2020), in the research undertaken, showed that environmental conditions, fertilization, and the culture substrate can influence both the characteristics of lettuce plants and their average mass.

Melad and Fahima (2018), based on some studies, showed that there were differences between the use of chemical and organic fertilizers, with the data obtained showing that in the case of organic fertilization, the average mass of lettuce heads was higher compared to the organically fertilized ones, studies were also carried out by Asmaa et al. (2021) and Micu et al. (2022).

Ayşe et al. (2005) analyzed the influence of substrates composed of zeolite and perlite as well as the nutrient solution on the growth of lettuce plants and found that the use of zeolite increased the nitrogen and potassium content.

The study conducted showed that the use of the biochar product as a growth substrate in lettuce culture led to an increase in production and quality performance (Singh et al., 2012; Smider, 2014; Noor et al., 2023).

MATERIALS AND METHODS

The study was carried out in the research greenhouses belonging to the Center for the Study of the Quality of Agro-Food Products within USAMV of Bucharest. The seedlings were produced in the greenhouse, and at planting, they were 27 days old, presenting an average of 3.3 true leaves. Planting was done on mats filled with perlite with a grain size of 4 mm, the mats having a size of 1 m long and a capacity of 10 L. The experimental variants consisted of the application of the nutrient solution with concentrations of 1.5 mS, 2.5 mS, and 3.5 mS. In the case of each variant of EC, three pH values were used, namely nutrient solutions with pH 5, 6 and 7 (Table 1).

Table 1. The experimental variants

	EC mS/cm^2		
	1.5	2.5.	3.5
pH	5	5	5
pH	6	6	6
pH	7	7	7

During the growth of the plants, the temperature, light, and CO_2 content in the culture space were constantly monitored. The growth of the plants in height, the formation of the number of leaves, and the diameter of the lettuce plants were monitored dynamically, and at the end of the culture, after 32 days from planting, the mass of the plants and the root volume were determined. Also, at the end of the culture, we recorded the values for each plant and interpreted how the pH of the solution, in accordance with the concentration of the nutrient solution, influenced the height growth of the lettuce plants, the number of leaves formed per plant, the diameter of the plants, the mass of the plants, and nitrate content. Influence of nutrient solution concentration on height growth of lettuce plants. All recorded parameters were analyzed using Two ways ANOVA, Statistical software version 10, Turkey test was proceeded to differentiate between the treatments at $p<0.05$.

RESULTS AND DISCUSSIONS

The temperatures recorded in the greenhouse were appropriate for the species, being between 19°C and 24.67°C with an average during the growth period of 22.3°C.

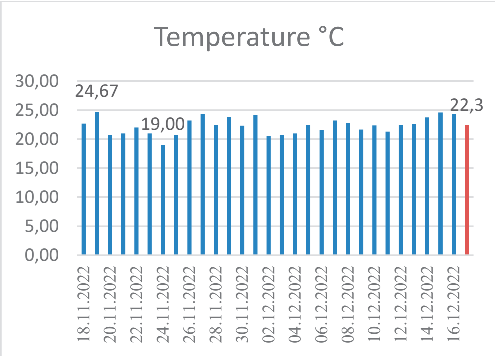


Figure 1. Temperature values recorded in the greenhouse during the culture period

The carbon dioxide concentration was on average 396.78 ppm, the maximum values recorded were 468.39 ppm and the minimum 337.85 ppm (Figure 2).

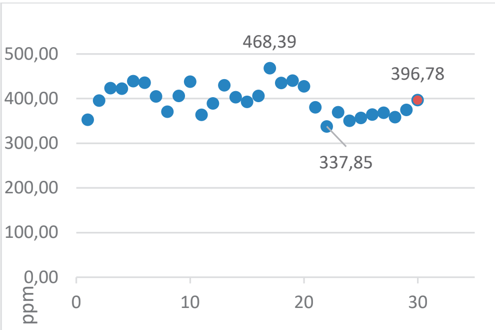


Figure 2. Carbon dioxide content recorded in the evening during the cultivation period

Figure 3. Appearance of the experiment in the greenhouse

From figure 4, it can be seen that at a pH of 5, in the case of all EC variants, the lowest plant height was obtained. In the case of the pH of 6 at the EC value of 1.5 mS/cm^2, the highest plant height (cm) was obtained, and in the case of using EC of 3.5 mS/cm^2, the plant height was also lower compared to the rest of the variants. The highest plant height values were recorded at pH 7, with plant height being the highest for all EC values.

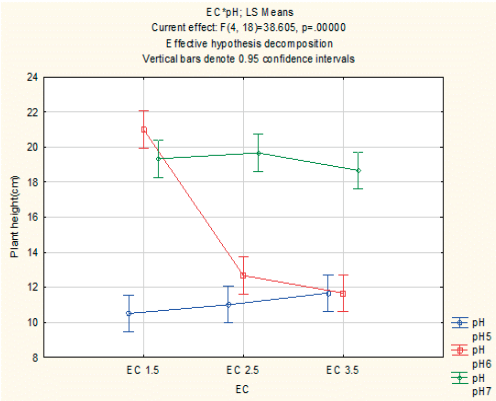


Figure 4 Influence of EC and pH on plant height

The number of leaves per plant recorded the highest values when using an EC of 1.5 mS/cm^2. Increasing the EC value to 2.5 and 3.5 mS/cm^2, respectively, did not lead to the formation of a large number of plant leaves, except for the use of EC 2.5 and at a pH of 7 (50.33 leaves/plant). The interaction between the EC used and the pH led to obtaining a lower number of leaves per plant in the case of a pH of 5 (Figure 5).

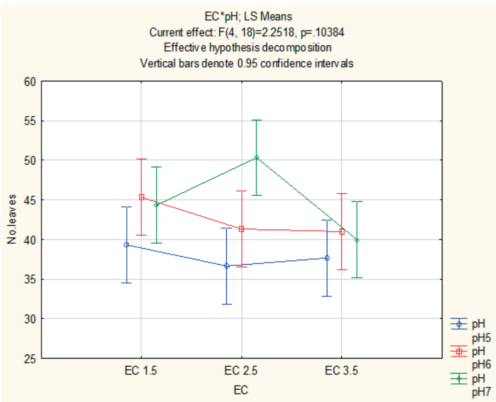


Figure 5 Influence of EC and pH on the number of leaves per plant

In the case of the interaction between EC and pH, a very significant influence was found on the growth in diameter of lettuce plants. The largest diameter of the plants was recorded when a pH of 7 was used. The smallest diameter of the plants was recorded when a pH of 5 was used. In the case of using a pH of 6, it was found that the largest diameter was obtained in the plants where the EC was 1.5 mS/cm^2 and the lowest in plants with an EC of 2.5 mS/cm^2 (Figure 6).

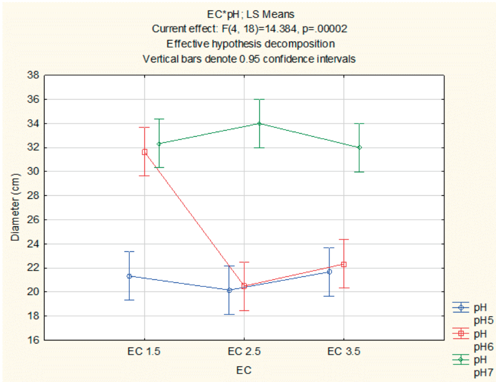


Figure 6. Interaction between EC and pH on diameter growth of lettuce plants

Based on the data analysis, we could find that in the case of using a pH value of 5, the average plant mass recorded the lowest values. In the case of all EC nutrient solution concentrations: 1.5 2.5 and 3.5 mS/cm^2) registering a very significant influence ($p = 0.0000$). In the case of using pH 7, it was found that the plant mass was the highest compared to the rest of the variants (Figure 7).

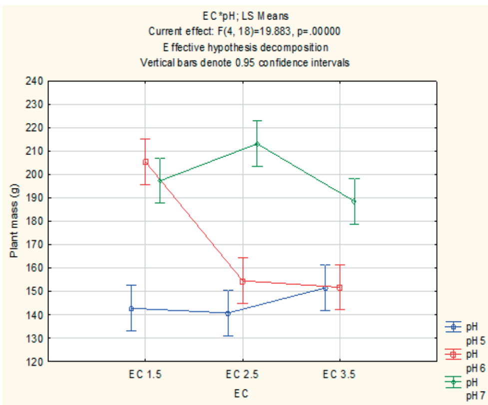


Figure 7. Interaction between EC and pH on plant mass

Analyzing the interaction between EC and pH values on the volume of the root system, it was found that there were no significant differences between its size (Figure 8).

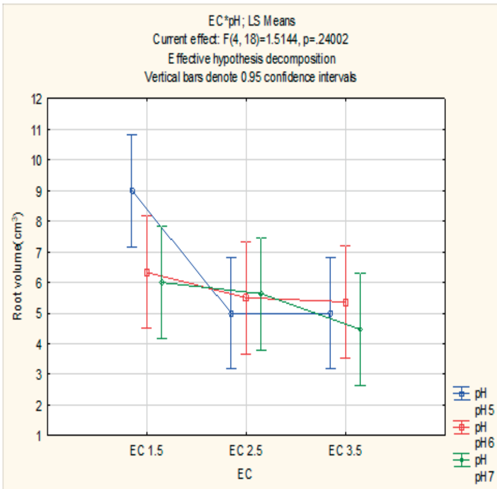


Figure 8. The interaction between EC and pH on root volume size

The correlation carried out to see the influence of the EC of 1.5 on the plant mass found a significant relationship (R^2 = 0.6406) which indicates that, in this situation, at a pH of 6 and 7 respectively, the plant mass increased (Figure 9).

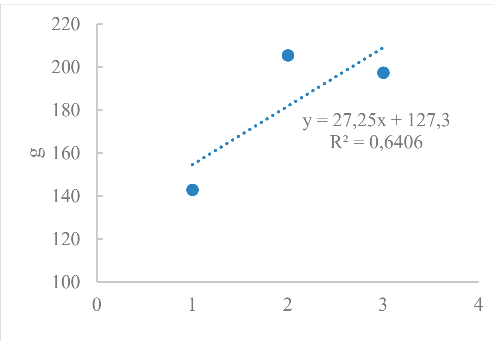


Figure 9. Relationship between EC of 1.5 mS/cm^2 and pH on plant mass

In the case of the EC of 2.5 mS/cm^2, a very significant correlation was also found in relation to the pH of the nutrient solution (R^2 = 0.8869). Also, and in the case of EC of 3.5 mS/cm^2 the relationship was significant (R^2 = 0.7551) (Figures 10 and 11).

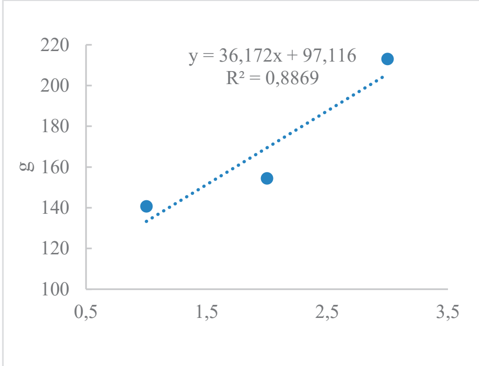


Figure 10. Relationship between EC of 2.5 and pH on plant mass

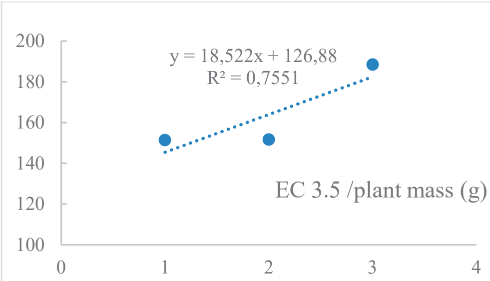


Figure 11. Relationship between EC of 3.5 mS/cm^2 and pH on mass of lettuce plants

Analyzing the influence of the pH, depending on the concentration of the nutrient solution (EC), it is noted that, in the case of using a pH of 5 and an EC with a value of 3.5 mS/cm^2, the mass of the plants was greater, which also emerges from the correlation performed (R^2 = 0.5833) (Figure 12).

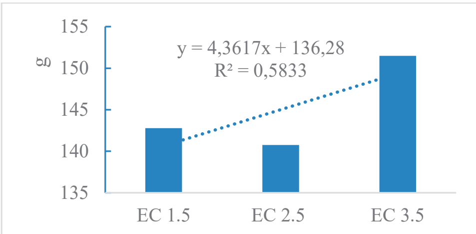


Figure 12. Correlation carried out at the pH 5 variant and EC values of the nutrient solution on plant mass

In the case of using a pH of 6, it was found that, using an EC of 1.5 mS/cm^2 led to a higher average mass, the correlation performed was negatively significant (R^2 = 0.789) as the EC increased (Figure 13).

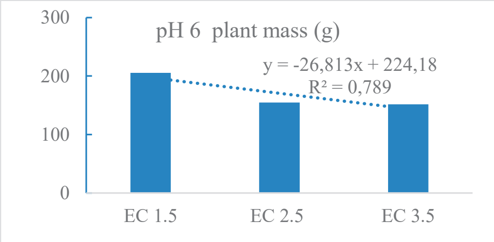


Figure 13. Correlation carried out at the pH 6 variant and the different EC values of the nutrient solution on plant mass

The correlation performed when using a pH of 7 led to the need to increase the EC to 2.5 mS/cm^2 to achieve a higher average plant mass. An EC of 3.5 mS/cm^2 and at a pH of 7 does not lead to an increase in production (R^2 = 0.1231) (Figure 14).

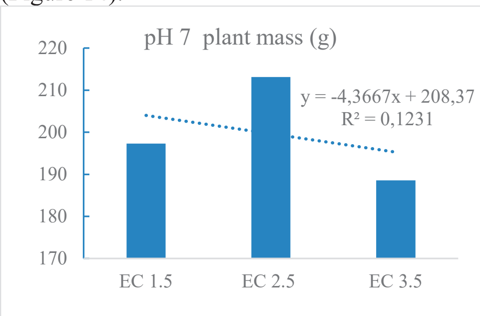
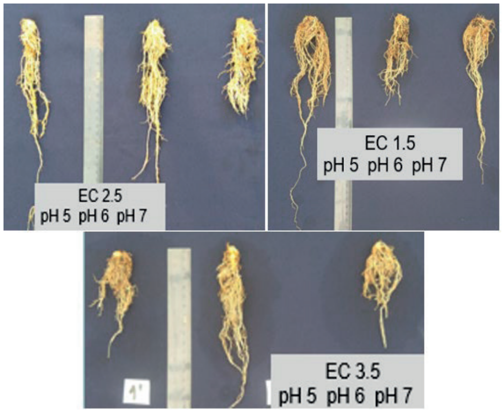


Figure 14. Correlation carried out for the variant of pH 7 and the EC of the nutrient solution on plant mass

Figure 15 shows an aspect from the moment of carrying out the determinations in the greenhouse.

Figure 15. Aspect from the time of carrying out the determinations in the greenhouse

a



b

Figure 16. Appearance of lettuce plants (a) and appearance of roots (b) on experimental variants

In summary, in Table 2 it is noted that the highest average plant mass was recorded at an EC of 1.5, as well as plant height, number of leaves, plant diameter and root volume. A very significant influence of the EC nutrient solution concentration and even of the pH is noted.
From the interaction of EC and pH, very significant differences were noted regarding the mass, height and diameter of the plants and insignificant differences between the number of leaves and root volume (Table 2, Figure 16).

Table 2. The influence of EC and pH on the analyzed parameters

	Plant mass(g/plant)	Plant height(cm)	No.leaves	Diameter(cm)	Root volume(cm3)
EC 1.5	181.8 ± 10.1a	16.9 ± 1.6a	43.0 ± 1.4a	28.4 ± 1.8a	7.1 ± 0.8a
EC 2.5	169.5 ± 11.2b	14.4 ± 1.4b	42.8 ± 2.5a	24.9 ± 2.4b	5.4 ± 0.3b
EC 3.5	163.9 ± 6.7b	14.0 ± 1.2b	39.6 ± 0.7a	25.3 ± 1.7b	4.9 ± 0.3b
EC	***	***	ns	***	*
pH 5	145.0 ± 2.7c	11.1 ± 0.2c	37.9 ± 1.5b	21.1 ± 0.6c	6.3 ± 0.9a
pH 6	170.5 ± 9.2b	15.1 ± 1.5b	42.6 ± 0.9a	24.8 ± 1.8b	5.7 ± 0.3a
pH7	199.6 ± 4.1a	19.2 ± 0.3a	44.9 ± 1.9a	32.8 ± 0.5a	5.4 ± 0.3a
pH	***	***	**	***	ns
EC x pH	***	***	ns	***	ns

CONCLUSIONS

Temperature and atmospheric humidity conditions in the greenhouse were the factors ~~those~~ corresponding to the species throughout the experiment.

The height of the plant was influenced by the value of the EC, but also by the pH, as result indicated that the height of the plants maximized at pH 1.5 and with pH 7.

The lowest plant heights were recorded when a pH of 5 was used.

The number of leaves was also influenced by the pH not only by EC, but the greatest variations being also recorded at a pH of 7.

The diameter of the plants was also influenced by the highest pH values, being recorded at a pH of 7. In the case of the concentration of the EC nutrient solution had an influence on the growth in diameter of the lettuce plants, and the plant responded well to EC 1.5 mS/cm^2. The mass of the plants was influenced by both the pH value and the EC value. The highest values were recorded under the conditions of using a pH of 7 and an EC of 1.5 mS/cm^2.

The root volume was influenced to a lesser extent by the pH value, as here too, but the largest root volume of the roots was recorded in the case of using an EC of 1.5 mS/cm^2.

The correlations performed indicated significant values in the case of the interaction between EC and pH.

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PROTECTION OF BAMBARA GROUNDNUTS (*VIGNA SUBTERRANEA*) FROM ROOT-KNOT NEMATODES: A CLIMATE SMART APPROACH

Nicholus MNYAMBO, Moses TIMANA, Mmagadima SEBATI, Lerato KGOTSE, Zakheleni DUBE

University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa

Corresponding author email: 201626411@ump.ac.za

Abstract

Bambara groundnut (BG) is third most important legume in sub-Saharan Africa. Currently, there are no commercial varieties, and reports of Meloidogyne species impacts have been made without empirical evidence. The withdrawal of synthetic nematicides has increased the demand for alternative products. Therefore, the objectives of this study were to determine the resistance status of BG varieties to M. incognita and potential use of Maerua angolensis extract in the control of M. incognita and growth of BG varieties. Screening experiment: six BG varieties were inoculated with ≈5000 M. incognita eggs and second-stage juveniles under greenhouse condition. Management experiment: 6 x 5 factorial experiment under similar conditions. The first factor consisted of retained BG varieties and the second factor was made up of M. angolensis extract levels, fenamiphos, and untreated control. At 130 days after inoculation, all the six varieties were hosts to M. incognita at varying degrees, but plant growth variables were not sensitive to the nematode. Plant extracts had no direct effects on nematode numbers but increased the BG varieties tolerance to nematode attack

Key words: *Bambara groundnut, host sensitivity, host status, Maerua angolensis, Nemacur.*

INTRODUCTION

Bambara groundnut (*Vigna subterranean*) is the third most important legume crop after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in Sub-Saharan Africa (Hillocks et al., 2012). Bambara groundnut yields in Africa are comparatively small, ranging between 650 and 850 kg/ha (Jada et al., 2011). Apart from genetic potential, diseases and pests such as nematodes are one of the key causes of low Bambara groundnut yields (Kankam and Adomako, 2014).

As a result, pest and disease management is an important criterion for increasing agricultural productivity of the crop (Dias et al., 2015). Although evidence suggests that the crop is not free of pests and diseases, as previously documented by Murevanhema and Jideani (2013), very little research has been undertaken on pests and diseases of Bambara groundnut. This has a negative impact on the crop's production. *Maerua angolensis* has been found to be effective against nematodes (Khosa et al., 2020). Investigating effective control techniques to reduce yield losses observed in small-scale farmer production systems is critical. Although chemical management with nematicides has proven to be the most effective, nematicides are often expensive and out of reach for small-scale farmers. Due to their harmful impact on the environment, non-target organism many nematicides have been taken off the agrochemical market. It is becoming more popular to identify and use local plant resources for nematode control (Khosa et al., 2020). Due to the growing problem of environmental pollution caused by the use of persistent pesticides, this strategy of managing *Meloidogyne* species is appealing (Kankam and Adomako, 2014.).

Several studies have demonstrated that plant extracts from several indigenous plants especially neem (*Azadirachta indica*) products are efficient against insects and nematodes (Khosa, 2021; Khosa, 2020; Mashela et al., 2017). *Maerua angolensis* plant extracts have been extensively reported to be effective in the management of *Meloidogyne incognita* on tomato plants (Khosa et al., 2021) but their effects on nematodes attacking traditional crops such as Bambara groundnuts has not been empirically studied. Hence, two studies were carried out to determine the host status and host sensitivity of Bambara groundnuts and to test the efficacy of *Maerua angolensis* in the treatment of *Meloidogyne incognita* on the same crop.

MATERIALS AND METHODS

The research was carried out under greenhouse conditions at the University of Mpumalanga, (25.4365° S, 30.9818° E), Nelspruit, South Africa. Greenhouse temperatures were set between 25 and 30°C, with thermostatically activated fans and wet-wall at opposite ends.

Screening trial: Thirty-centimetre diameter plastic pots were placed on greenhouse benches at the spacing of 0.3 m intra- and inter-row spacing. Six Bambara groundnut varieties (‘DP-W’, ‘BR-W’, ‘BS-W’, ‘CW-W’, ‘BB-W’, and ‘DB-W’), were then planted in separate pots. Four seeds of each variety were separately planted in each pot. At two true-leaf-stage, the seedlings were thinned to one plant per pot. A week after thinning, Bambara groundnut seedlings were inoculated with 5000±20 *M. incognita* eggs and second-stage juvenile (J2) using a 20 ml plastic syringe by putting them on cardinal stem points in a 2½ cm deep hole. A randomized complete block design (RCBD) with ten replicates was used to arrange the treatments.

Management trial: The location and experimental setup were as described previously in screening trial, except that treatments consisted of *M. angolensis* extract leaf powder levels applied on different varieties. A week after inoculation of seedlings with nematodes, treatments of medicinal plant meal extracts were spread thinly by hand around the base of seedlings on the soil surface and then lightly mixed with topsoil. The experiment constituted of a 6 x 5 factorial arrangement, the first factor being six Bambara groundnut varieties and the second factor consisted of three *M. angolensis* leaf powder levels; (5, 10 and 15 g/pot that translated to 697, 1394, and 2091 kg/ha, respectively) plus a standard synthetic, commercial nematicide, Nemacur® 10GR (a.i. fenamiphos) at a rate of 697 kg/ha, as well as a negative control (plants inoculated with nematode without any treatment). The treatments were also arranged in an RCBD, with five replications.

DATA COLLECTION

Plant variables: At 56 days after inoculation, plant height was measured from the crown to the end of the flag leaf, stems were cut at the soil line, and stem diameter estimated at 3 cm above the cut end using a manual vernier caliper (Model no: 464-9952, RS PRO, South Africa). The chlorophyll content was measured using the chlorophyll meter (Minolta spad-502, Hangzhou, China). Shoots were weighed and then dried in an oven for 72 hours at 70 °C. The root systems were taken up from the soil, washed to eliminate any soil particles, blotted dry, and weighed.

Nematode variables: The nematodes were extracted from the entire root system using the maceration and blending process (Fourie et al., 2017). The soil in each pot was well mixed, and 250 mL of soil samples were collected, with nematodes extracted from it using a sugar floatation and centrifugation process (Fourie et al., 2017). Eggs and J2 from root and J2 from the soil were counted under a stereomicroscope (Olympus Corporation Tokyo 163- 0914, CX23RTFS2) from a 1 ml aliquot of each sample. The final nematode population density (Pf) was calculated by adding total eggs and J2 from the root system to total J2 from the soil. Reproductive potential (RP = total eggs and J2 in root/fresh root mass) and Reproductive factor (RF = final nematode population (Pf) divided by the initial nematode inoculated (Pi)) indicators of nematode's ability to reproduce were computed (Kayani and Mukhtar, 2018).

DATA ANALYSIS

Plant growth and nematode data were subjected to analysis of variance (ANOVA) using Statistix 10 software. Prior to ANOVA, data were subjected to Shapiro-Wilk normality test for homogeneity determination. Non-continuous data were transformed using log10(x+1) to homogeneous variance. Means of significantly different variables were separated using the Fishers’ least significant difference (LSD) test at 0.05 level.

RESULTS AND DISCUSSIONS

For the screening experiment: There were no significant differences ($P > 0.05$) in all measured nematode variables and plant growth variables among varieties, except for plant runner length which was highly significant ($P \leq 0.01$). The reproductive potential and reproductive factors were also not significantly different among cultivars, while there were all greater than one. Variety 'CW-W' had the longest runners compared to all other varieties, which were not different from each other (Figure 1).

For the management experiment: the first-order interaction between *Maerua angolensis* plant extracts and Bambara groundnut landraces were highly significant ($P \leq 0.01$) for all nematode variables measured (Table 1). There were no different in the effectiveness of the plant extract levels on nematodes population densities. Plant extract levels reduced total nematodes in roots. However, plants treated with Nemacur® had lower total nematodes in roots compared to plant treated with plant extract. Nemacur® significantly reduced all nematode variables in all Bambara groundnut landraces compared to plants exposed to plant extracts, except landrace 'DB-W' where Nemacur® effects on the total nematodes were not significantly different to those of plant extracts. Nematodes reproduced and increased in numbers in all plants treated with all the different plant extracts level, however, Nemacur reduced the total nematodes in all varieties except var. 'DP-W', 'CW-W' and 'DB-W' were nematodes increased in numbers. Plant extracts had no direct effects on nematode numbers but increased the Bambara groundnut varieties tolerance to nematode attack.

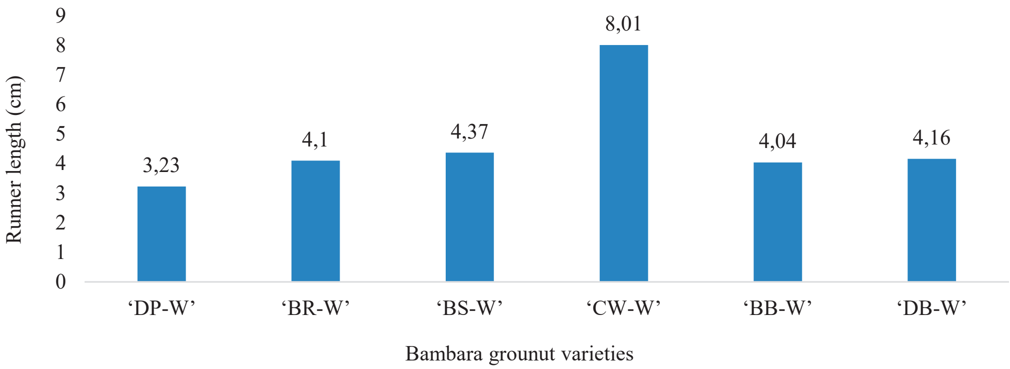

Figure 1: Effects of Bambara groundnut varieties on runner length

Table 1. Interactive effects of Bambara ground variety and *Maerua angolensis* plant extracts on *Meloidogyne incognita* variables

	Plant extract														
	Total nematode in roots					Juveniles in soil					Total nematode				
Variety	0^{x}	5	10	15	Nemacur®	0	5	10	15	Nemacur®	0	5	10	15	Nemacur®
'DP-W'	3.27^{cdef} (1950)	3.39^{abcdef} (4770)	3.50^{abcdef} (5610)	3.25^{cdefg} (2400)	2.26^{hi} (600)	4.24^{ab} (175000)	4.50^{a} (32000)	4.30^{a} (26000)	4.61^{a} (50000)	3.08^{cd} (6000)	4.29^{abcd} (19450)	4.63^{ab} (43770)	4.48^{abc} (34610)	4.70^{a} (57400)	4.03^{bcd} (11600)
'BR-W'	4.00^{a} (15450)	3.26^{cdef} (2160)	3.39^{abcdef} (4470)	3.32^{bcdef} (4320)	1.92^{ij} (225)	4.27^{a} (23000)	4.18^{ab} (19000)	4.49^{a} (38000)	4.56^{a} (38000)	0.00^{e} (000)	4.66^{a} (49450)	4.32^{abcd} (25160)	4.56^{ab} (46470)	4.73^{a} (54320)	1.92^{f} (225)
'BS-W'	3.97^{ab} (13950)	3.72^{abcd} (9000)	3.74^{abcd} (7890)	3.31^{bcdef} (2520)	1.54^{j} (75)	4.55^{a} (38000)	4.42^{a} (40000)	4.62^{a} (59000)	4.68^{a} (50000)	2.22^{d} (3000)	4.71^{a} (51950)	4.57^{ab} (55000)	4.79^{a} (73890)	4.70^{a} (52520)	2.54^{e} (3075)
'CW-W'	2.59^{gh} (450)	3.33^{abcdef} (2880)	3.52^{abcde} (4260)	3.43^{abcdef} (3330)	2.88^{efgh} (750)	4.62^{a} (46000)	4.40^{a} (28000)	4.31^{a} (23000)	4.54^{a} (41000)	3.24^{bc} (16000)	4.76^{a} (59450)	4.51^{abc} (33880)	4.47^{abc} (33260)	4.64^{ab} (51330)	3.84^{d} (16750)
'BB-W'	3.86^{abc} (8775)	3.49^{abcdef} (4680)	3.57^{abcd} (9240)	3.16^{defg} (2100)	1.54^{j}(75)	4.19^{ab} (16000)	4.49^{a} (34000)	4.64^{a} (56000)	4.37^{a} (26000)	2.22^{d} (3000)	4.55^{ab} (35775)	4.65^{a} (46680)	4.78^{a} (72240)	4.57^{ab} (39100)	3.07^{e} (3075)
'DB-W'	2.86^{fgh} (750)	3.38^{abcdef} (2490)	3.26^{cdef} (3150)	3.64^{abcd} (6420)	3.35^{abcdef} (3375)	4.24^{ab} (18000)	4.54^{a} (45000)	4.44^{a} (43000)	4.49^{a} (32000)	3.24^{bc} (10000)	4.25^{abcd} (18750)	4.63^{ab} (57490)	4.57^{ab} (55150)	4.61^{ab} (41420)	3.89^{cd} (16375)
F-value			3.70					2.40					3.66		
$LSD_{0.05}$			0.6642					1.0103					0.6158		
P-value			0.0000					0.0019					0.0000		

[x]Column means followed by the same letters are not significant different at $P \leq 0.05$ according to Fisher's Least Significant Difference.
[y]Values in brackets are untransformed means [$\log_{10}(x+1)$].

The host status of a plant to nematodes is usually determined by any one or both of the following indicators, reproductive potential and reproductive factor (Anwar and McKenry, 2010). The reproductive factor of the nematode, quantifies the nematode reproduction through a ratio of initial versus final nematode populations on the plant, making this a perfect indicator under a closed environment where soil nematodes can be manipulated (Kayani and Mukhtar, 2018). When the reproductive factor on a particular host is less than one, it indicates that the nematode cannot reproduce on that host hence that plant is not a host (Kayani and Mukhtar, 2018). If the reproductive factor is greater than one, the nematode has multiplied successfully on the host (Pofu et al., 2010). The reproductive factor is a basic indicator of nematode reproductive potential and hence gives a good indication of a plant's level of resistance (Steyn et al., 2012).

On the other hand, the reproductive potential indicator uses the ratio of nematodes in roots as a function of the total fresh root mass (Anwar and McKenry, 2010). The reproductive potential of a nematode can be used as an indicator of whether a plant is susceptible, tolerant, or resistant to the presence of nematode. Susceptible plants are those that will be affected by the presence of nematodes and result in plant growth failure, while a tolerant plant is able to withstand the presence of nematode and continue to grow without being affected (Jones et al., 2013). Resistant plants restrict the presence of nematode at all, if nematodes are exposed to such plants they will not feed at all (Ralmi et al., 2016). According to Pofu et al. (2020) judgement of host status indication, all the Bambara groundnut varieties in the current study were a host of *M. incognita*. *Meloidogyne incognita* was able to penetrate and multiply in all six varieties (Steyn et al., 2012). Muhamman et al. (2010) reported Bambara groundnut to be good host for *Meloidogyne javanica*, another *Meloidogyne* species. The current study hence expands on the host spectrum of Bambara groundnuts to nematode species. Varieties having lower reproductive factors will be appropriate for the management of root-knot nematodes (Mukhtar and Hussain, 2019).

On host sensitivity, the current study reports that nematodes had no effect on the plant growth variables between the inoculated and un-inoculated plants of the same variety, and varieties also had similar growth trends except for var. 'CW-W' which had longer runner lengths. The similarity in the growth variables of the varieties when exposed to nematodes and when nematodes are absent is an indication of low sensitivity (Pofu et al., 2010). *Meloidogyne incognita* is a major problem on Bambara groundnut, according to Kwerepe and Labuschagne (2004), who screened 15 Bambara groundnut landraces from Botswana and South Africa and found signs of resistance and tolerance to this nematode. The performance of South African varieties performance in the presence of nematodes contradicts reports made by Imegwu et al. (2014) and Ogbuji (1979), who observed stunted growth in Bambara groundnut types from Nigeria inoculated with a local population of *M. incognita* and *M. javanica*. Tobih et al. (2011) reported runner length as a good sign of a healthy plant able to utilize the soil nutrients optimally. Previous studies by McDonald and De Waele (1989) suggested that *M. javanica*-tolerant Bambara groundnut variety exists.

Bambara groundnut length runner is important in determining the yield of the crop because it is where the pods are formed even though there is no documented literature on the relationship between the two.

Mukhtar et al. (2014) and Mukhtar et al. (2013) reported that variation in plant response can be due to the genetic make-up of the host plant and different environmental factors. Karikari (2000) reported that varieties with a growth behavior like var. 'CW-W' have the potential for strategic usage as an intercrop, where it can provide faster ground cover and help restrict weed growth by spreading out. Variety 'CW-W' is known by the fact that it produces more flowers and matures earlier than other types (Berchie et al., 2010). In Bambara groundnuts, it is believed that the number of runner lengths is a good indicator of high productivity since flowers and pods are formed in the runner length. There is currently a scarcity of knowledge on the relationship between floral characteristics and pod production in Bambara groundnut. This information is critical for understanding the pod formation ability of the Bambara groundnut, as well as designing the optimum selection method(s) for increasing pod production in the crop.

Following the environmental concerns produced by chemical control measures, the use of diverse sections of indigenous plants as botanical extracts has been increasingly essential in pest management in recent years (Mamun and Ahmed, 2011) It is apparent from the result of this study that plants with plant extract levels showed the best plant growth variables (stem diameter, runner length, fresh root mass, dry shoot mass, and high chlorophyll content) and development compared to plants treated with Nemacur®. According to Khosa et al. (2020), a higher shoot weight caused by the presence of *Marua angolensis* was observed in the years 2008 and 2011.

The lower rate of nematode multiplication in Nemacur® treated plants could be due to a lower rate of juvenile penetration into varieties, which would restrict nematode multiplication and result in a low fecundity rate (Mukhtar et al., 2013). Mnyambo et al. (2021) reported Nemacur® consistently reducing nematode densities in roots of all six cultivars. The current results contradict work by Khosa *et al*. (2020) who reported *M. angolensis* suppressing the nematode population densities significantly by 87 to 88%. Khosa et al. (2021, 2020), observed suppression of *M. incognita* caused by *M. angolensis* similar to the suppression caused by Nemacur® in 2008 and 2009. Moreover, *M. angolensis* did not suppress nematode densities in 2011 (Khosa et al., 2020), which supports the current study. Khosa et al. (2020) reported that the increase in plant growth variables caused by the presence of *M. angolensis* can result in more roots for the nematodes to reproduce in numbers.

The continuing growth of the plant without being compromising the presence of nematode is a sign that Bambara groundnut is tolerant to *M. incognita*. The influence of plant extracts results in improved root health, active root nodules for active nitrogen fixation, enhanced root anchorage, and good uptake of nutrients and water from the soil by the plant.

The improvement of plant growth variables of plants treated with *M. angolensis* and *T. elegans* have been explained mostly using three phenomenon, firstly that the ability of plant extract to suppress nematodes results in plants growing in an environment of pest free hence better performance (Ugwuoke, Ukwueze and Ogwulumba, 2011). The second phenomenon, is that addition of plant extracts improves the soil conditions around the plants' rhizosphere (Khan et al., 2019). The improvement of soil conditions has received extensive attention in agronomic studies of organic amendments and disease suppression (Khan et al., 2019). The third concepts which is quickly gaining attention is the induction of resistance and/or defense reactions by compounds produced by plant extracts in treated plants (Thakur and Sohal, 2013). The concept of induced resistance is well studied in plant fungal and bacterial diseases, with elicitors being identified as inducers of such resistance (Thakur and Sohal, 2013). Induced plant resistance has been defined as the enhancement of a plant's defense against pests through extrinsic physical or chemical stimuli and these stimuli could be a pathogen, insect herbivore or wounding, beneficial microbe, or chemical agent (De Kesel et al., 2021). A further study could need to be conducted to comprehensively explain the relationship between Bambara groundnut varieties and *M. angolensis*.

The improved plant growth observed in plants treated with plant extract could be due to that more nutrients were readily available to the plants, which no doubt promoted vigorous growth to counteract the nematode's attack (Nworgu, 2006). The study is supported by the work of Khosa et al. (2020) who observed an increase of plant growth variables in plants treated with *M. angolensis*. According to the current findings, *Maerua angolensis* plant extract did not effectively control nematode population in Bambara groundnut, but did improve measured plant growth variables.

CONCLUSIONS

The use of crop varieties exhibiting resistance against pathogenic nematodes is important for limiting nematode populations in soils. Bambara groundnut varieties responded differently to the presence of nematode. All

Bambara groundnut varieties tested were a host to *Meloidogyne incognita* with nematode able to reproduce. Moreover, all tested Bambara groundnut showed a level of tolerance to the nematode. This was observed when the plant growth variables were not affected by the presence of nematode. In this study, the use of these extracts to control nematode population densities in Bambara groundnut was not effective, but Nemacur® effects differed per variety.

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PRELIMINARY RESULTS OF FISH FERTILIZER EFFECTS ON LETTUCE

Ailin MOLOȘAG[1, 2], Violeta Alexandra ION[1], Oana Cristina PÂRVULESCU[3], Aurora DOBRIN[1], Oana-Crina BUJOR[1], Andrei MOȚ[1], Viorica LAGUNOVSCHI-LUCHIAN[2]

[1]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[3]Chemical and Biochemical Engineering Department, National University of Science and Technology POLITEHNICA Bucharest, 1-7 Gheorghe Polizu Street, Bucharest, Romania

Corresponding author email: violeta.ion@qlab.usamv.com

Abstract

To address the latest environmental and pollution challenges, as well as the growing need for food due to a progressively expanding population, this study proposes an alternative approach to increase food production by testing residual materials from the blue value chain for their potential use in farming and agriculture. For this purpose, the experimental framework involved establishing a greenhouse potted lettuce crop with the curly variety 'Simona'. We considered four experimental variants, two of which involved organic fertilization treatments using fish residues, i.e., cod bone powder (F1) and common ling bone powder (F2), one with mineral fertilizer (F3), and a control (C). The experimental monitoring period was set from November 14, 2022, to January 4, 2023. The lettuce crop was evaluated for physiological and chemical characteristics. Fish-based fertilizers showed results similar to those of the variant with mineral fertilizer, but superior to the control variant. Consequently, fish-derived organic fertilizers could be an effective alternative to mineral fertilizers for greenhouse-grown lettuce.

Key words*: greenhouse, lettuce, fertilizer, blue bioeconomy.*

INTRODUCTION

According to the United Nations, the global population exceeded 8000 million by the end of 2022. World population growth is exponential, and according to the FAO, 870 million people in this population do not have enough food to eat (Oluwole et al., 2023).

This problem is compounded by environmental issues, with agriculture leading to environmental pollution at all levels: air, soil, and water. In agriculture and horticultural production, water is consumed in significant quantities (Liu et al., 2022) and is a focal point because some countries are experiencing a severe water deficit or have even reached 'Day Zero' (Chen et al., 2019; Millington & Scheba, 2020). The primary agricultural water pollutants are mineral nitrogen and pesticide used extensively in plant protection and fertilization, often applied excessively (Savcı, 2012).

New European policies aim at addressing these concerns, *e.g.*, through promoting soil health and organic growing (EU2020). This includes increased interest in the use of various types of organic waste, such as fish residues, manure, sewage sludge, etc. These efforts are strengthened by bioeconomy and circular economy concepts, which may increase sustainability and preserve biodiversity by replacing mineral inputs with adverse effects (Løes & Adler, 2019; Ahuja et al., 2020; Coppola et al., 2021; Cardarelli et al., 2023).

Fish-based fertilizers can be one example of a bioeconomic solution, where residual materials which are currently wasted, may replace mineral fertilizers which are getting scarce (phosphorus) and/or require high energy consumption (nitrogen). By-products from the fish processing industry contain valuable nutrients, making a compelling case for their use as fertilizers (Illera-Vives et al., 2015). Furthermore, the use of fish remains is not a new concept. Ahuja et al. (2020) noted their historical utilization by Egyptians, Incas, and Mayans, as well as their traditional application in coastal crop cultivation. Fish meal produced in Norway has a modern counterpart in the form of commercial fertilizer products (Ahuja et al., 2020). These products are formulated using various fish-derived components and are approved for use in certified organic farming (Illera-Vives et al., 2015). It is necessary to develop a stable formula for the processing of such residues to ensure that the derived fertilizers do not have harmful effects on crops, including issues like phytotoxicity and organic microcontaminants, which can have metabolic and phenotypic implications for the plants (Matamoros et al., 2021). These processed fish residues can be found in various forms, *e.g.*, emulsion, hydrolysate, compost, digestate (Ahuja et al., 2020).

Fish residual materials have been successfully used as fertilizers in various forms and within different culture systems. Some of the most popular methods include aquaponics (Cohen et al., 2018) and hydroponics (Ahmed et al., 2021). Fish emulsions and hydrolysates are typically used in liquid form, but dried, powdered fish residues also showed positive results when used for biosolarization (Zou et al., 2023), and had significant growth effects in recent field experiments (Løes et al., 2022). Illera-Vives et al. (2015) reported positive results using compost from fish residue and seaweed on tomato and lettuce as a second crop. They recommended composting as one of the most cost-effective methods for stabilizing waste materials. Xu & Mou (2017) concluded that fish-derived protein hydrolysates can enhance lettuce growth, increase leaf water content, boost leaf chlorophyll content, and improve gas exchange. Muscolo et al. (2022) achieved favourable results in the cultivation of Tropea red onion (*Allium cepa*) using an extract from solid residues of anchovy fillet waste compared to commonly used mineral and organic fertilizers.

Fish-based fertilizers offer a sustainable solution for the future, and their applicability can be scalable with the right formulation. However, despite their many benefits, there are some impediments, including high salt content, which is a challenge for the growth of various plant species, particularly lettuce, known for its sensitivity to elevated salt levels. In this context, the aim of the study was to assess the impact of fish powder fertilizers on potted lettuce growth in a protected indoor environment (greenhouse).

MATERIALS AND METHODS

Experiment design

The experiment was conducted in the research greenhouse at the Research Center for Food Quality and Agricultural Products, USAMV Bucharest. We chose a Romanian variety of curly lettuce, 'Simona', which was started from seeds purchased from a local market.

The experimental treatments, related to the types of fertilizers used, included: (C) Control, using peat substrate (OPM 540 W, Kekkilä-BVB, Finland) and perlite; (F1) Peat amended with cod (*Gadus morhua*) bone powder; (F2) Peat amended with common ling (*Molva molva*) bone powder; (F3) Peat amended with a commercial mineral fertilizer.

The characterization of the fertilizers used in the current experiment can be found more detailed in our previous paper (Moloșag et al., 2023). The amounts of fish-based fertilizers were 30 g/pot of cod bone powder and 40 g/pot of common ling bone powder, and these fertilizers were used only at the beginning of the experiment, when the substrate was prepared.

The experiment began by marking the pots and preparing the substrate, using a peat/perlite ratio of 4/1 (v/v). After thorough mixing, the substrate was evenly distributed among 40 pots of 3 L each, with 10 pots designated for each fertilizer variant (C, F1, F2, and F3). The required amount of fertilizer for each variant was then added and thoroughly mixed. The materials and relevant stages of the experimental procedure are shown in Figure 1. Initially, each pot was watered with 1000 mL of tap water to moisten the peat. Before the seedlings were transplanted, a total of 2.8 L of water was applied to the pots based on their appearance and moisture requirements. The substrate was prepared 51 days before the seedlings were transplanted to allow the fish powder to partially degrade. During this period the substrate was kept relatively moist, and its pH values after 51 days were as follows: 5.90±0.02 for C, 6.94±0.03 for F1, 7.02±0.02 for F2, and 6.23±0.02 for F3.

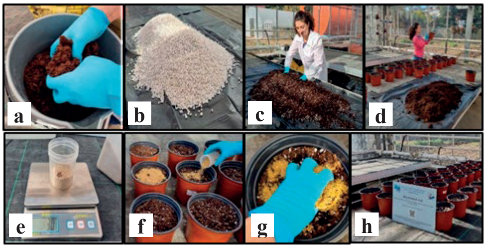

Figure 1. Peat (a); perlite (b); preparation of peat/perlite substate (4/1 v/v) (c); adding the substrate to the pots (d); weighing the fish-based fertilizer (e); adding the fertilizer over the substrate (f); mixing the fertilizer with the substrate (g); pots containing mixtures of fertilizer and substrate (h)

Lettuce seedling experiments

On October 18, 2022, the seeds of the commercial lettuce variety 'Simona' were sown in the control substrate. The seeds emerged after three days, corresponding to code BBCH 09, which indicates cotyledons breaking through the soil surface. On October 28, 2022, when the first true leaf was unfolded (Figure 2d), corresponding to BBCH code 11, the seedlings were transplanted into the 72-cell tray (Figure 2a-c). On November 14, 2022 (initial time, T_0), when the third true leaf was unfolded (Figure 2e), corresponding to BBCH code 13, the seedlings were transplanted (1 plant/pot) into the experimental pots (Figure 2f). The newly transplanted plants were watered with 200 mL water/pot and the selected growth parameters started to be monitored. The experiment lasted until January 4, 2022 (final time, T_f).

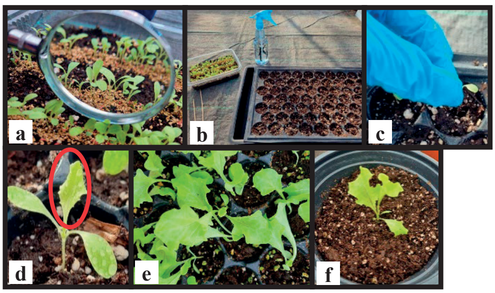

Figure 2. Lettuce seedlings grown in the peat/perlite substrate (28/10/2022) (a); 72 cell tray containing peat/perlite substrate (4/1 v/v) (b); transplanting lettuce seedlings into cells (28/10/2022) (c); first true leaf (31/10/2022) (d); third true leaf (14/11/2022) (e); lettuce seedlings transplanted into pots (14/11/2022) (f)

Substrate monitoring and characterization

The following parameters of the substrate were measured in the greenhouse: substrate moisture (*M*, %) using a MO750 Soil Moisture Meter (EXTECH Instruments, Nashua, NH, USA), electrical conductivity (*EC*, mS/cm) and temperature (*t*, °C) using a HI-98331 Groline Direct Soil Conductivity (EC) & Temperature Tester (Hanna Instruments Smithfield, RI, USA).

Substrate samples were monitored for total carbon content (*TC*, %) and total nitrogen content (*TN*, %) using the Dumas method (Moț et al., 2022) and measured with an EA 3100 elemental analyzer (Eurovector, Pavia, PV, Italy). Dry matter (*DM*, %) was determined using a Memmert Universal Oven (Memmert, Schwabach, Germany).

Plant monitoring and characterization

The number of leaves (*NL*) and plant height (*H*, cm) were monitored in the greenhouse (Drăghici et al., 2016). To calculate the specific leaf area (*SLA*, cm^2/g), which is the ratio of leaf area (*LA*, cm^2) to dry matter content (*DM*, g), lettuce leaf analyses were performed using WinFolia software (LA2400, Regent Instruments, Sainte-Foy, Quebec, Canada) and an Epson Expression 11000 XL scanner to obtain *LA* values, along with a Memmert Universal Oven (Memmert, Schwabach, Germany) for *DM* measurements.

Total carbon content (*TC*, %) and total nitrogen content (*TN*, %) in the leaves were determined using the Dumas method (Moț et al., 2022) and measured with an EA 3100 elemental analyzer (Eurovector, Pavia, PV, Italy).

The analyses were carried out in the laboratories of the Research Center for Studies of Food Quality and Agricultural Products at the start and end of the experiment.

Statistical analysis

The data were analyzed with IBM SPSS statistical software, and Duncan's Multiple Range Test was applied to determine if the means of selected parameters for the four experimental variants were significantly different ($p < 0.05$) or not (Moț et al., 2021).

RESULTS AND DISCUSSIONS

Substrate characterization

The lettuce crop faced unfavourable temperature conditions with temperatures soaring up to 31°C in the greenhouse, hindering its growth. In this study, crops were cultivated in a greenhouse using natural light. Nevertheless, the distribution of light within the greenhouse was influenced by various factors including nearby structures and seasonal changes. The light distribution also influenced the substrate moisture content (*M*). Despite being irrigated based on its needs, the control substrate exhibited lower *M* levels (9.86±0.91%) at the end of the experiment (M_f) compared to the variants containing fish fertilizers, which had *M* values of 21.63±1.58% for F1 and 20.88±5.55% for F2 (Table 1). These values are notably lower compared to those reported by Martins et al. (2023), who recorded substrate moisture values exceeding 70%. However, they are similar to the findings of Matamoros et al. (2021) for the organic fraction of municipal solid waste (OFMSW) used as a substrate in their experiment, which had a moisture value of 23%.

Electrical conductivity (*EC*) represents the total ion concentration of a solution (Samarakoon et al., 2020). It can hinder plant growth and development when its values do not meet the plant requirements. Numerous studies have been conducted to determine the most suitable *EC* for growing lettuce, particularly in a hydroponic system. The studies concluded that lettuce was moderately sensitive to salinity (Andriolo et al., 2005; Ünlükara et al., 2008; Martins et al., 2023), with greater sensitivity observed in the early stages of development (Martins et al., 2023). In our investigation, *EC* values (Table 1) varied for the fish-fertilized variants for T_0, showing significantly different values for F1 (0.79±0.12 mS/cm) and F2 (1.08±0.27 mS/cm) compared to the control (0.12±0.02 mS/cm). Data summarized in Table 2 indicate a significant decrease in *EC* for F1 and F2 at the end of the experiment. Martins et al. (2023) reported *EC* values of 2.44-2.79 mS/cm and even above 3.2 mS/cm in the early stage of one of the experiments, without the lettuce seedlings showing visual symptoms of excess salts.

Abou-Hadid et al. (1996) concluded that fresh weight yield decreased with an increase in *EC* of the nutrient solution in a hydroponic system. They also determined that the microelements in lettuce plants were affected not only by *EC* levels but also by variety. Samarakoon et al. in 2019 found that the optimum lettuce yield was obtained at 1.8 mS/cm for several cultivars in two growing seasons.

The mean values of substrate temperature specified in Table 1, *i.e.*, 20.9-22.6°C, are normal in the greenhouse.

Carbon and nitrogen are extremely important for the proper development of plants (Cardarelli et al., 2023). Carbon is found in different forms, and the available carbon, in the appropriate doses, can regulate excess nitrate in the soil, but an oversupply of it can create soil hypoxia, which is detrimental to plant growth or even lethal (Qin et al., 2019). Data summarized in Table 2 highlight a decrease in total carbon content (*TC*) and an increase in total nitrogen content (*TN*) of the substrate at the end of the experiment. The most significant difference between the mean values of *TC* was for T_0-F2 (40.00±1.07%) and T_f-F2 (32.85±0.67%).

Table 1. Moisture content, electrical conductivity, and temperature of the substrate for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*M* (%)	*EC* (mS/cm)	*t* (°C)
T_0-C	$11.26±1.88^{a}$	$0.12±0.02^{ab}$	$21.9±0.2^{bc}$
T_0-F1	$21.9±2.00^{cd}$	$0.79±0.12^{d}$	$21.8±0.4^{b}$
T_0-F2	$24.13±2.34^{d}$	$1.08±0.27^{e}$	$22.1±0.2^{c}$
T_0-F3	$9.21±1.54^{a}$	$0.07±0.02^{a}$	$22.6±0.5^{b}$
T_f-C	$9.86±0.91^{a}$	$0.08±0.02^{a}$	$20.9±0.1^{a}$
T_f-F1	$21.63±1.58^{c}$	$0.33±0.12^{c}$	$21.2±0.3^{a}$
T_f-F2	$20.88±5.55^{c}$	$0.32±0.18^{c}$	$21.1±0.2^{a}$
T_f-F3	$17.89±2.94^{b}$	$0.21±0.07^{bc}$	$21.1±0.3^{a}$

Different letters in the same column indicate a significant difference ($p < 0.05$).

Table 2. Total carbon content and total nitrogen content of the substrate for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*TC* (%)	*TN* (%)
T_0-C	$40.20±0.29^{d}$	$0.71±0.05^{a}$
T_0-F1	$37.58±0.98^{c}$	$1.33±0.05^{bc}$
T_0-F2	$40.00 ±1.07^{d}$	$1.40 ±0.15^{c}$
T_0-F3	$37.62 ± 0.43^{c}$	$0.68±0.05^{a}$
T_f-C	$37.73±0.25^{c}$	$1.25±0.00^{b}$
T_f-F1	$37.04±0.22^{c}$	$1.69±0.03^{d}$
T_f-F2	$32.85±0.67^{a}$	$1.42±0.01^{c}$
T_f-F3	$35.27±0.20^{b}$	$1.38±0.16^{bc}$

Different letters in the same column indicate a significant difference ($p < 0.05$).

Plant characterization

The number of lettuce leaves (*NL*) showed significant differences for all fertilized variants compared to the control at the end of the experiment (Table 3). At the end of the experiment, F1 showed the highest value (19.9±2.2) from the fertilized variants compared to those of F2 (17.6±2.5) and F3 (18.2±3.7). Our results are similar to those of Islam et al. (2021) and very slightly different from those reported by Xu & Mou (2017).

In terms of the plant height (*H*), significant differences were for all fertilized variants between the initial and final time, but not between variants (Figure 3). However, the highest value was recorded for F3 (18.9±3.9 cm) and the lowest for C (9.0±1.6 cm). The values of this growth indicator obtained by Vetrano et al. (2020) in a floating system were higher, varying between 23.6 cm and 27.4 cm, depending on the treatment applied. Also, *H* values obtained in this study are similar to those reported by Islam et al. (2021) for *Lactuca sativa* cv. Green Wave (18.63 ± 1.8 cm) and *Lactuca sativa* cv. New Red Fire (16.95 ± 0.76 cm) grown in a mixture of soil (20%), vermicompost (40%), and spent mushroom compost (40%).

Specific leaf area (*SLA*) or specific foliar area (*SFA*) is a representative element when referring to the plant growth (Schneider et al., 2018). It is an indicator of the plant photosynthetic capacity, explaining growth variations influenced by the action of different environments, the latter also impacting leaf density and/or thickness (morphological traits) (Liu et al., 2016; de Ávila Silva et al., 2021). A study focused on salt stress tolerance showed that lettuce leaf morphology, represented by petiole thickening, changed under stress conditions, *i.e.*, *SLA* value decreased (Vetrano et al., 2020). But when taking into account the transplant quality, a low *SLA* value indicates a superior quality of the plant material (Spalholz & Hernández, 2018). Regarding *SLA* (Table 3), significant differences were between T_0 and T_f and between fertilizer variants and control variant for T_f. Vetrano et al. (2020) reported a mean *SLA* value of 703.2 cm^2/g in the unstressed lettuce plants and Schneider et al. (2018) obtained a mean value of 400 cm^2/g. Xu & Mou (2017) concluded that fish-derived protein hydrolysates influenced positively leaf juiciness and leaf water content but had no effect on *SLA*, the latter having values between 302±18 cm^2/g for the control and 330±13 cm^2/g for the variants where the treatment was applied. Salinas et al. (2019) in their attempt to quantify changes in biomass accumulation of lettuce following amino acid treatments obtained results similar to ours.

In terms of total carbon and nitrogen contents of lettuce leaves (*TC* and *TN*), significant differences were recorded between T_0 and T_f (Table 4). Tabulated data highlight a decrease in *TC* and an increase in *TN* at the end of the experiment, when the lowest levels of *TC* and *TN* were for F1 (32.58±0.19%) and F2 (5.78±0.04%), respectively.

Table 3. Number of leaves, plant height, and specific leaf area of lettuce for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*NL*	*H* (cm)	*SLA* (cm^2/g)
T_0-C	3.4±0.5^a	8.3±0.8^a	12.17±3.63^a
T_0-F1	3.6±0.5^a	8.7±0.5^a	12.17±3.63^a
T_0-F2	3.8±0.4^a	8.0±0.6^a	12.17±3.63^a
T_0-F3	4.0±0.0^a	8.8±1.1^a	12.17±3.63^a
T_f-C	4.6±0.5^a	9.0±1.6^a	44.37±14.93^b
T_f-F1	19.9±2.2^c	18.8±2.7^b	99.48±40.04^c
T_f-F2	17.6±2.5^b	18.3±2.0^b	107.12±33.71^c
T_f-F3	18.2±3.7^b	18.9±3.9^b	108.48±11.28^c

Different letters in the same column indicate a significant difference ($p < 0.05$).

Table 4. Total carbon content and total nitrogen content in the plant leaves for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*TC* (%)	*TN* (%)
T_0-C,F1,F2,F3	36.53±0.54^d	5.40±0.20^a
T_f-C	34.13±0.22^c	6.18±0.10^{cd}
T_f-F1	32.58±0.19^a	6.02±0.06^c
T_f-F2	33.40±0.08^b	5.78±0.04^b
T_f-F3	34.57±0.46^c	6.37±0.10^d

Different letters in the same column indicate a significant difference ($p < 0.05$).

CONCLUSIONS

The mean values of selected lettuce growth parameters *i.e.*, number of leaves (*NL*), plant height (*H*), and specific leaf area (*SLA*), for fertilizer variants (F1, F2, and F3) were significantly higher than those for control variant (C). The mean value of *NL* for F1 (19.9) was significantly higher than those for F2 (17.6) and F3 (18.2), which were similar, whereas the mean values of *H* (18.3-18.9 cm) and *SLA* (99.48-108.48 cm^2/g) for all fertilizer variants were similar.

Considering the results obtained for the variants fertilized with fish residues compared to conventional fertilization we can conclude that fish-based fertilizers could partially replace the mineral fertilizers and can be a more efficient and environmentally friendly alternative. However, further studies with better controlled environmental conditions and monitoring of more parameters are needed.

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RESEARCH ON THE DYNAMICS OF METEOROLOGICAL PHENOMENA IN THE SOUTH OF OLTENIA AND THE ESTABLISHMENT OF THE SUITABILITY OF THE SWEET PEPPER CULTURE FOR THE CURRENT CLIMATIC CONDITIONS

Alina-Nicoleta PARASCHIV[1], Aurelia DIACONU[1], Ștefan NANU[1], Daniela Valentina POPA[2]

[1]SCDCPN Dăbuleni, 217 Petre Baniță Street, Călărași, Dolj, Romania
[2]University of Craiova, Faculty of Horticulture, 13A.I. Cuza Street, Craiova, Romania

Corresponding author email: alina22paraschiv@yahoo.com

Abstract

In order to highlight the amplitude of climate changes in the southwest of Romania, at Dăbuleni Research - Development Station for Plant Culture on Sands (SCDCPN Dăbuleni), the climatic data from the archives of the own meteorological station were analyzed and processed over a period of 65 years. The dynamics of meteorological phenomena in the period 1956-2020 was analyzed, a sufficiently long period for a meaningful analysis and correct conclusions. Data processing from the 780 months and more than 23,400 data records was the source of our conclusions. Helman`s criterion was used to determine the types of thermal and pluviometric time, and as normal climatic averages the averages of the measurements of the climatic parameters of the 65 years were used, the period being significantly extended. The thermal norm in the Dăbuleni area is much higher than in most of Oltenia with monthly summer averages between 21.6°C in June, 23.6°C in July and 23.0°C in August, and the seasonal average summer of 22.8°C. Summer heat produces staged forcing of the sweet pepper crop and early and massive ripening.

Key words: *climate change, temperature, precipitation, sweet pepper.*

INTRODUCTION

The town of Dăbuleni, an important vegetable basin for the south-western area of Romania and beyond, is located in the vast area of sandy land in the south of Oltenia (>100000 ha) included between the towns: Calafat, Poiana Mare, Sadova, Bechet, Dăbuleni and Danube River, area generically named "Oltenia's Sahara". At the beginning of the 9th century, along with the intensive development of agriculture, a vast action of cutting Romania's forests was triggered in order to create agricultural land. Along with this agricultural development action and the cutting of forests, the specific problems of soil degradation also started. The sandy lands uncovered by vegetation quickly became fragile, and the action of the wind created a huge space with dunes, which in dry years, under the action of wind deflation, became "moving". In the decade 1960-1970, vast works were carried out to improve the agricultural land and build a particularly important irrigation system in this area based on the main source of water, the Danube River. As a result, the once unproductive area was transformed into a fertile land where more than 60 species of plants were cultivated, and the improvements included protective forest curtains that mitigated the force of the wind and stopped the blowing of snow in during blizzards, creating a reserve of water in the soil needed in the spring, at the initiation of vegetative processes in agricultural crops (Diaconu et al., 2019).

The destruction of the irrigation system, protective curtains and other land improvements, which occurred after 1989, quickly restored the situation existing before 1960. The global warming occurred in this area progressively manifested itself, thus the first monthly maximum temperature value ≥40.0°C was recorded on 1985 July 31, 40.8°C, and subsequently the frequency of occurrence of exceptional temperature values increased during the summer, as a result of the increase in the frequency of heat waves and their intensity (Marinică I., 2006).

According to the prevailing landforms, Oltenia is characterized by a great diversity of topoclimates, which develop on the background of climatic floors, in the amphitheater, with southern exposure. (Bogdan, 2001; Marinică I., 2006).

The Dăbuleni vegetable basin is included in the plain topoclimate, with the elementary topoclimates of valleys, meadows, fields, sand dunes and with specific topoclimatic phenomena: temperature inversions, minimum temperatures below -30°C and maximums above 35-40°C, precipitation in the form of showers and maximum amounts in 24 hours of more than 300 l/m², with dry and hot winds in summer and dry and drought phenomena (Bogdan, 1996; Popa et al., 2015). The areas with sandy soil in Oltenia contributed to the creation of the warm topoclimate of this area through the properties and way of interaction with solar radiation of this type of soil. The sandy surface heats up quickly and strongly under the influence of solar radiation, and from it the air heats up strongly and quickly. The albedo of sand is 0.40, a very high value compared to that of other types of soil (Marinică & Marinică, 2016). The thermal conductivity properties of the sand, which has interstitial spaces in its structure, cause its strong heating in a superficial layer, and in depth the temperature remains favorable for agricultural crops. The intense heat, on hot days, causes a strong and rapid evaporation of water from the superficial layers, reducing the water reserve available from the soil and an intense transpiration of the plants, thus producing their wilting (the reduced water reserve specific to the wilting coefficient is reached). Thus, the soil water reserve is conditioned by the rapid drainage of water from the soil due to its structure (Sandu et al., 2010; Bogdan et al., 2020). Due to the geological conditions, in the depth of the soil, an impermeable rock layer is present, as an extension of the foundation of the Pre-Balkan Plateau in northern Bulgaria, under the Danube in the Oltenia Plain. As a result, in the underground of this area there are water phreatic layers that can provide sufficiently high flows for irrigation, and the continuous water supply of these layers is produced by the rich flow of the Danube, but also by other phreatic layers that come from under the Hills Oltenia, fed in turn by the precipitation, which falls all year round in the area of Oltenia. As a result, the Dăbuleni area can be favorable for agricultural crops of all types. Under irrigated conditions, cereals, technical plants, sugar beet and a wide range of vegetable plants, plants of very high food value, can be grown successfully.

MATERIALS AND METHODS

The SCDCPN Dăbuleni, since its foundation, has been equipped with a weather station, the first in this area, established 4 years before the one in Bechet, which is part of the modern national network of weather stations of Romania. As a result, the climatic data from the data archive of the meteorological station of the SCDCPN Dăbuleni were analyzed and processed (coordinates 43°48′04″N, 24°05′31″E). Correlations were made with the data from the meteorological station in Bechet, which belongs to the National Meteorological Administration (coordinates 43°47`N, 23°57`E) located at 11.4 km (according to GPS). Climate data from 1956-2020 was analyzed, so a period of 65 years, long enough for meaningful analysis and correct conclusions. The processing of data from the 780 months and more than 23,400 data records contributed to the conclusions of this paper.

The methodological bases of the processing of these data were focused on the climatic criteria presented in the following tables:

Table 1. Types of weather according to the deviation (ΔT°C) of daily air temperature averages from normal values (N) (Hellmann-type criterion)

ΔT°C	Weather feature	Abbreviation
>10°C	Too hot	**EC**
7.0…10.0°C	Wery warm	**FC**
4.0…6.9°C	Hot	**C**
2.1…3.9°C	Slightly warmer	**UC**
-2.0…+2°C	Thermally normal	**N**
-3.9…-2.1°C	Slightly cooler	**UR**
-6.9…-4.0°C	Cold	**R**
-10.0…-7°C	Very cold	**FR**
<-10°C	Too cold	**ER**

(Source: MARINICĂ and MARINICĂ, 2016).

Table 2. Hellmann's criterion for mean monthly temperatures

Deviation of temperature monthly averages compared to normal (°C)	**Characterization**	**Abbreviation**	**Coloring**
≥ 10°C	Too hot	**EC**	**EC**
5°C…9.9°C	Wery warm	**FC**	**FC**
2.0°C…4.9°C	Hot	**C**	**C**
1.0°C …1.9°C	Slightly warmer	**Cl**	**Cl**
-0.9°C…+0.9°C	Thermally normal	**N**	**N**
-1.9°C …-1.0°C	Slightly cooler	**RC**	**RC**
-4.9°C…-2.0°C	Cold	**R**	**R**
-9.9°C…-5°C	Very cold	**FR**	**FR**
≤-10°C	Too cold	**ER**	**ER**

Table 3. Hellmann's criterion for average seasonal and annual temperatures

Average seasonal or annual temperature deviation relative to normal (°C)	**Characterization**	**Abbreviation**	**Coloring**
≥ 5°C	Too hot	**EC**	**EC**
2.6°C…4.9°C	Wery warm	**FC**	**FC**
1.1°C…2.5°C	Hot	**C**	**C**
0.6°C …1.0°C	Slightly warmer	**Cl**	**Cl**
-0.5°C…+0.5°C	Thermally normal	**N**	**N**
-1.0°C …-0.6°C	Slightly cooler	**RC**	**RC**
-2.5°C…-1.1°C	Cold	**R**	**R**
-4.9°C…-2.6°C	Very cold	**FR**	**FR**
≤-5°C	Too cold	**ER**	**ER**

Table 4. Rainfall weather types according to Hellmann's criterion

Applied to monthly rainfall amounts			Applied to annual rainfall amounts		
Types of time	Deviation (%)	Abbreviation	Types of time	Deviation (%)	Abbreviation
Excessively Rainy	>50	**EP**	Excessively Rainy	>20	**EP**
Very Rainy	30.1…50.0	**FP**	Very Rainy	15.1…20.0	**FP**
Rainy	20.1…30.0	**P**	Rainy	10.1…15.0	**P**
A little rainy	10.1…20.0	**PP**	A little rainy	5.1…10.0	**PP**
Normal	-10.0…+10.0	**N**	Normal	-5.0…+ 5.0	**N**
A little dry	-20.0…-10.1	**PS**	A little dry	-10.0…-5.1	**PS**
Dry	-30.0…-20.1	**S**	Dry	-15.0…-10.1	**S**
Very Dry	-50.0…-30.1	**FS**	Very Dry	-20.0…-15.1	**FS**
Excessively Dry	>-50.0	**ES**	Excessively Dry	>-20.0	**ES**

Helman's criterion was used to determine the types of thermal and pluviometric time, and as normal climatic averages the averages of the measurements of the climatic parameters of the 65 years were used, the period being significantly extended.

RESULTS AND DISCUSSIONS

Air temperature regime in the Dăbuleni area

The Dăbuleni area is placed in an area with a strong influence of the sub-Mediterranean climate, with frequent warm air advections in the cold and warm seasons and with frequent higher temperatures than in most of Oltenia. Winters are generally warm, springs are early with summer days and tropical in some years starting from the end of March, summers excessively hot, and autumns long and mild with monthly averages of 18.0°C in September, 11.7°C in October and 5.6°C in November and positive minimums, which also extends into December. This warm area of Oltenia is characterized by the fact that only in January the multiannual monthly average is negative, and in the other months it is positive or slightly negative (≥-0.1°C).

Average monthly temperature values (normal) in the analyzed interval (1956-2020) were between -1.4°C in January and 23.6°C in July, the warmest month of year, and the annual average of 11.7°C (Table 5).

Table 5. Characteristic values of air temperature (°C) for the interval 1956-2020 from the meteorological station of SCDCPN Dăbuleni

Month	I	II	III	IV	V	VI	VII
Average	-1.4	1	6	12.5	17.6	21.7	23.6
CMMavg	5.7	7.4	10.0	17.8	22.4	24.8	27.7
DCMMavg	2007	1958	2017	2018	1958	2002	2002
Cmmavg	-9	-7.8	0.9	8.3	9.3	18.6	21.1
DCmmavg	1963	1956	1957	1997	1985	1976	1979
TminAbs	-30.5	-27	-16.9	-3.7	1	5.8	8.5
DataTminAbs	1963;25	2012;1	2018;1	2012;2	2000;5	1962;9	1987;28
CMMmin	-1.9	-1.6	-1.2	4	10.6	16.1	22
DataCMMmin	1988;18	2013;5	2014;11	2004;4	2003;17	2003;22	2020;9
Avgmin	-14.1	-12.2	-6.1	0.2	6	10.2	12
TMAXAbs	19.6	24.3	28.9	34.7	37.5	43.3	43.5
DataTMAXAbs	2007;21	2016;22	1983;26	1998;6	1993;28	2007;26	2007;24
cmmTMax	3.2	4.0	12.1	17.6	26.7	28.4	32.5
DatacmmTMax	1996;3	2003;21	1996;30	2002;30	1957;x	2011;24	1969;5
Avgmax	11.3	15.0	22.6	27.2	31.6	34.9	36.7
Month	VIII	IX	X	XI	XII	Year	
Average	23	18	11.7	5.6	1	11.7	
CMMavg	26.3	21.4	18.8	9.7	9.4	13.5	
DCMMavg	2003	1994	1962	2019	1960	2019	
Cmmavg	18.4	14.3	8.6	-0.7	-6.3	9.2	
DCmmavg	1976	2006	1972	1993	1962	1985	
TminAbs	6	-2.2	-6.7	-20	-22	-30.5	
DataTminAbs	1981;30	1977;30	1988;28	1993;26	1997;18	1963;25	
CMMmin	16.1	13.0	5.9	0.6	-0.7	-9.4	
DataCMMmin	2016;14	1999;7	1999;18	2002;7	1987;11	2020;8.I	
Avgmin	10.9	5.4	-1.1	-5.7	-10.5	-17.5	
TMAXAbs	42.6	38.0	34.8	28.8	20.5	43.5	
DataTMAXAbs	2012;24;26	2007;28	1991;1	1989;1	1957;x	2007;24:VII	
cmmTMax	23.4	25.0	20.1	2.6	4.5	33.6	
DatacmmTMax	2009;4	2002;8	2010;2	1998;5	1963;6	2002;16.VII	
Avgmax	36.2	32.7	27.3	19.1	13.5	37.8	

(Average = monthly temperature average (or annual for annual column); CMMavg = highest monthly temperature average; DCMMavg = record year of highest monthly temperature average; Cmmavg = lowest monthly temperature average; DCmmavg = year of record of lowest monthly average temperature; TMinAbs = absolute minimum temperature; DataTminAbs = record date of absolute minimum monthly temperature (year;day); CMMmin = highest monthly minimum temperature; DataCMMmin = date of record of highest minimum temperature (year;day); Avgmin = average of monthly minimums; TMAXAbs = absolute maximum monthly temperature; DataTMAXAbs = record date of absolute maximum monthly temperature (year;day); cmmTMax = lowest maximum temperature monthly; DatacmmTMax = record date of the lowest monthly maximum temperature (year; day); Avgmax= average of monthly maximums; x= missing data).

The lowest monthly temperature averages ranged from -9.0°C recorded in January 1963 (the year when one of the coldest winters was recorded on the European continent) to 21.1°C recorded in July 1979 (Table 5).

The highest monthly temperature averages were between 5.7°C in January 2007 (the winter of 2006-2007 was the warmest winter on the European continent and holds the climatic record of the warmest winter for Romania as well) and 27.7°C in July 2002.

According to the average monthly temperature values, the warmest months were: January 2007 with an average of 5.7°C, February 1958 with an average of 7.4°C, March 2017 with an average of 10.0°C, April 2018 with average 17.8°C, May 1958 average 22.4°C, June 2002 average 27.7°C, August 2003 average 26.3°C, September 1994 average 21.4°C, October 1962 average of 18.8°C, November 2019 with an average of 9.7°C and December 1960 with an average of 9.4°C. With the exception of 5 months (February 1958, May 1958, September 1994, October 1962 and December 1960), the warmest months have been recorded since 2000, thus confirming the increase in climate warming after 2000. Since 2000, the criteria for evaluating the types of thermal weather have changed in accordance with the new climatic developments due to the increase in the frequency of long hot periods. Among the spring months, April 2018 holds the climate record of the highest temperature averages in the entire history of climate observations, not only in Romania but in a good part of the northern hemisphere. The highest average and maximum temperatures in the entire history of climate observations were recorded in July 2007.

The main vegetation period of the sweet pepper is from May 10-15 to October 1, with a maximum development in the months of July-August-September, when the peak of production is expected. The sweet pepper seedlings are obtained in the greenhouse, and the sowing date is between March 10-20, depending on the type of spring, and the latter depends on the type of winter (warm, cold, etc.). The germination time of sweet pepper seeds (duration in days from sowing to emergence) is long compared to other plants, about 3 weeks. Planting in the field is carried out depending on the climatic conditions, between May 10-15. In the Dăbuleni area, springs are usually early and warm, which makes the development of crops have optimal climatic conditions. The absolute climatic record of the earliest spring was recorded in the spring of 2016 with the absolute spring index of 583.1 (calculated according to the temperature values from the weather station in Bechet. Summers are particularly hot with intense heat waves, with a large number of tropical days and nights, autumns are long and dry, and the summer heat extends into September and heat waves also occur in the first 15-20 days of September. In winter, particularly cold air advections can occur from the Pre-Balkan Plateau (during cold waves) or from the mountainous area of northern Oltenia. The phenomenon of thermal inversion is frequent, especially in the cold season, but it also occurs in the warm season. Only the month of January has the multiannual average of negative temperature (-1.4°C), and the other months have positive averages. According to the annual temperature averages, the warmest year was 2019 with an average of 13.5°C, the year in which air temperature records were achieved at many meteorological stations in Romania, and in Dăbuleni the maximum temperature was 38.4°C.

The warmest years by mean annual temperature values ≥12.0°C have been recorded, with only four exceptions, since 2000, with annual means of: 1960 with annual mean of 12.5°C, 1966 with annual mean of 12.1°C, 1994 with annual average of 12.6°C, 1999 with annual average of 12.2°C, 2000 with annual average of 13.0°C, 2001 with annual average of 12.6°C, 2002 with annual average of 12.8°C, 2003 with annual average of 12.2°C, 2004 with annual average of 12.3°C, 2005 with annual average of 12.2°C, 2007 with annual average of 13.32°C, 2008 with annual average of 12.2°C, 2009 with annual average of 12.1°C, 2012 with annual average of 12.6°C, 2013 with annual average of 12.7°C, 2014 with annual average of 12.29°C, 2015 annual average of 12.9°C, 2016 annual average of 12.8°C, 2017 annual average of 12.9°C, 2018 with annual average of 13.1°C, 2019 with annual average of 13.5°C and 2020 with annual average of 13.3°C. It should be noted that since 2018, the averages have been ≥ 13.0°C. The increase in climate warming has been particularly rapid since 2000. Since 1990, most of the old climate records have been broken, and climate warming has accelerated since 2000, a process that continues today. The coldest years by annual average temperature with means ≤11.0°C were: 1963 with annual average of 10.7°C, 1969 with annual average of 10.3°C, 1973 with annual average of 10.8°C, 1976 with annual average of 10.4°C, 1980 with annual average of 10.6°C, 1982 with annual average of 10.9°C, 1983 with annual average of 10.9°C, 1985 with annual average of 9.2°C (the coldest year in the entire data set), 1986 with an annual average of 10.6°C, 1993 with an annual average of 10.9°C, 1997 with an annual average of 10.8°C, and 2011 with an average of 10.98°C. After 2011, such values were no longer recorded. For the entire analyzed period (1956-2020) the alternation of the percentage weights of thermal time during the vegetation period of the sweet pepper is shown in the Table 6 and graph in Figure 1.

Table 6. Matrix of types of thermal weather at Dăbuleni in the growing season (May-September) and adjacent months (April, October) of sweet pepper for the interval 1956-2020, calculated using Hellmann's criterion (TC = warm time = Cl+C+FC+EC; TR = cool time = RC+R+FR+ER, TN = normal thermal time)

Year	IV	V	VI	VII	VIII	IX	X
1956	CL	N	N	N	C	CL	N
1957	N	RC	C	CL	N	CL	N
1958	N	C	CL	C	C	CL	CL
1959	CL	N	N	C	N	RC	RC
1960	N	N	N	N	CL	RC	C
1961	CL	RC	N	RC	RC	N	N
1962	N	CL	RC	N	CL	N	FC
1963	N	N	N	N	CL	CL	N
1964	N	RC	N	N	RC	RC	CL
1965	R	N	N	N	R	CL	RC
1966	CL	N	R	N	N	N	C
1967	N	N	R	N	N	N	N
1968	C	C	N	N	R	N	RC
1969	RC	C	RC	R	N	N	RC
1970	N	RC	N	RC	RC	N	RC
1971	N	N	RC	R	RC	RC	RC
1972	C	N	CL	N	RC	R	R
1973	N	N	N	N	RC	N	N
1974	R	RC	RC	N	N	CL	N
1975	N	CL	N	RC	R	CL	N
1976	N	N	R	RC	R	RC	RC
1977	RC	N	RC	N	RC	R	R
1978	RC	RC	RC	RC	R	R	N
1979	RC	N	N	R	R	N	R
1980	RC	R	N	N	R	RC	CL
1981	RC	RC	N	RC	RC	N	CL
1982	R	N	N	RC	RC	CL	N
1983	CL	FR	R	N	RC	N	R
1984	R	N	R	N	R	N	C
1985	N	FR	RC	RC	N	RC	R
1986	N	N	RC	RC	RC	N	RC
1987	RC	RC	N	N	RC	C	RC
1988	R	N	N	CL	N	N	R
1989	C	RC	R	RC	N	RC	N
1990	N	N	N	N	RC	RC	N
1991	RC	R	N	N	R	N	N
1992	N	N	N	RC	C	RC	N
1993	RC	N	CL	N	N	N	CL
1994	CL	N	N	N	N	C	N
1995	N	N	N	N	N	N	N
1996	RC	C	CL	N	N	R	N
1997	R	CL	N	N	RC	RC	R
1998	CL	N	CL	CL	CL	RC	N
1999	CL	N	CL	CL	N	CL	N
2000	C	C	C	CL	C	N	N
2001	N	N	RC	CL	C	N	CL
2002	R	C	C	C	N	N	N
2003	RC		C	N	C	N	RC
2004	N	N	N	CL	N	CL	CL
2005	RC	N	N	N	RC	N	C
2006	CL	N	CL	N	C	R	N
2007	C	C	C	C	N	RC	N
2008	CL	N	N	RC	C	N	N
2009	N	N	N	N	N	N	N
2010	N	N	N	N	CL	N	R
2011	N	RC	N	N	N	C	RC
2012	CL	N	CL	C	CL	CL	C
2013	CL	C	N	N	CL	N	N
2014	N	RC	RC	N	N	N	N
2015	N	CL	RC	CL	CL	C	N
2016	C	N	CL	CL	N	C	N
2017	N	N	C	CL	CL	C	CL
2018	FC	C	N	N	C	CL	CL
2019	N	N	CL	N	C	C	CL
2020	N	N	N	N	CL	C	CL
TC%	29,2	20	24,6	21,5	30,8	30,8	26,2
TN%	41,6	58,1	49,2	52,3	32,3	41,5	44,6
TR%	29,2	21,9	26,2	26,2	-36,9	27,7	29,2

The predominance of normal thermal time (TN) is observed in the vegetation season, the normal here meaning much warmer than in other agricultural areas in Oltenia, which actually means exceptionally favorable climatic conditions from a thermal point of view for the culture of sweet pepper.

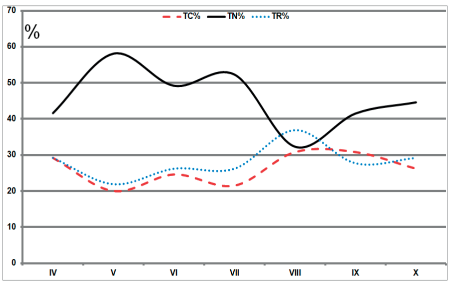

Figure 1. The alternation of the weight of thermal time types (TC%, TN%, TR%) in the Dăbuleni area during the sweet pepper vegetation period. (Source: Data processed from the data archive of the meteorological station of SCDCPN Dăbuleni)

The analysis of the amounts of precipitation recorded in the growing season of the sweet pepper in the interval 1956-2020, shows that the multiannual average (normal) is 270.3 l/m² (normal for vegetation season - SV). The lowest value was 79.8 l/m² (the climate record for the lowest amount of precipitation for the growing season) recorded in the dry year 1958, and the highest 541.0 l/m² (the climate record for the most large amounts of precipitation in the growing season) in the rainy year 1957 (Figure 2).

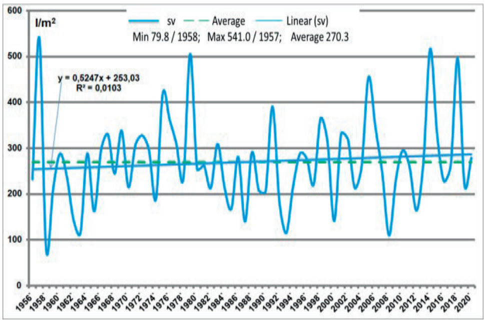

Figure 2. The variation of the annual amounts of precipitation (l/m²) in Dăbuleni in the interval 1956-2020

The rainiest growing seasons (with precipitation values ≥350 l/m²) were in the years: 1957 with 541.0 l/m², 1975 with 419.1 l/m², 1976 with 362.7 l/m², 1979 with 505.6 l/m², 1991 with 390.9 l/m², 1998 with 364.9 l/m², 2005 with 455.0 l/m², 2014 with 516.9 l/m² and 2018 with 495.9 l/m². The driest growing seasons (with precipitation values ≤ 150 l/m²) were in the years: 1958 with 79.8 l/m², 1962 with 143.5 l/m², 1963 with 114.8 l/m², 1987 with 140.0 l/m², 2000 with 141.4 l/m² and 2008 with 110.0 l/m².

CONCLUSIONS

It can be concluded that in the interval of 5 months from May to September, the sweet pepper crop completes its vegetative cycle almost entirely, except for the stage before being put in the field and what can still take place as a result of the extension of the warm weather in October. The aridification of the climate of the area is progressive and excessively hot summers lead to the need to irrigate the culture of sweet peppers, and the water reserves in the basement will be sufficient as long as the Danube flows and as long as the Oltenia Hills exist, the topoclimate here being the product of several factors that interconditions. In situations with prolonged and intense heat, shading nets are useful, which reduce the intensity of solar radiation and allow enough light to reach the plants for photosynthesis processes. The variability of the climate is particularly high, with rapid transitions in the spring from rainy and cool weather to hot and dry weather, and in the first month of autumn or at the end of August, rainy periods settle in some years. The thermal norm in the Dăbuleni area is much higher than in most of Oltenia with monthly summer averages between 21.6°C in June, 23.6°C in July and 23.0°C in August, and the seasonal average summer of 22.8°C. Summer heat produces stadial forcing of the sweet pepper crop and early and massive ripening. September's average of 18.0°C is 4 tenths of a degree higher than May, thus extending the warm weather and favorable conditions into September as well. The increasing trend of air temperature parameters (maximum, minimum, average) determines the translation of the summer season to autumn and the extension of favorable conditions in the first month of autumn. Climate warming also manifests itself in the Dăbuleni area in all months of the year. Deficient rainfall time prevails throughout the growing period of the sweet pepper, and for the entire vegetation period the percentage of deficient rainfall time is 46.2%, of normal 20.0%, and of excess rainfall 33.8%.

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IMPACT OF DIFFERENT IRRIGATION REGIMES ON CABBAGE GROWTH

Vera PETROVA, Viktor BICHEV

University of Forestry, Faculty of Agronomy, 10 Kliment Ohridski Blvd, Sofia, Bulgaria
Corresponding author email: vera_zamfirova@abv.bg

Abstract

Water is essential for agricultural production and food security. At present and in the future, irrigation will be carry out in condition of water shortage.
The field experiments were carry out in 2022 on the Experimental Field of the University of Forestry, located in the Sofia Field, near Vrajdebna village. The soil type is alluvial - meadow with light mechanical composition. The growing crop is a late season cabbage variety Balkan which is the standard variety for Bulgaria. Six variants of irrigation regime was tested, as follow 130%, 100%, 70%, 40% of irrigation rate, without first irrigation during the development stage and without second irrigation during the head formation. Climate, soil moisture, biometric data and yield was measure. The data show the greatest yield in variants without second irrigation. Furthermore, the results show that early drought stress effects can be compensate by an appropriate water supply in later growing stages.

Key words: *irrigation regime, drought stress, drip irrigation.*

INTRODUCTION

Bulgaria is located in the temperate continental climate zone, characterized by warm summers and cold winters. According to National Institute of Hydrology and Meteorology report, a temperature increase in Bulgaria between 2 and 5 degrees at the end of the 21st century. The available data for a long period show that the temperature sums during the growing season of the main agricultural crops are relatively stable, its mean that they are not a limiting factor for normal plant development (Stoyanova, R., 2020). However, this is not the case with precipitation. Unsustainable rainfed, predetermination the irrigation as the main factor for obtaining high and sustainable crops yields. Therefore, is need development of efficient and economical irrigation is the key for effective use of limited water resources.

With about 338 genera and more than 3,700 species, the *Brassicaceae* family is one of the major angiosperm families. Cabbage vegetable crops are grown for their valuable nutritional qualities. According to FAO data, about 2.6 million hectares of cabbage are cultivated in the world, from which about 55 million tons of fresh produce are obtained.

Irrigation of late cabbage has a much greater importance for the quantity and quality of the yield. Cultivation of late cabbage can be successfully carried out even with a deficit irrigation regime. In addition, a deficit irrigation strategy is a practice allows crops to maintain some degree of water deficit with insignificant yield loss (Abdelkhalik, A., 2019). This has the potential to increase water use efficiency and save water. It is expected that deficit irrigation would be applied to a wide variety of crops and in more regions, especially in arid and semiarid climates around the world.

In literature, there are a large number of studies aimed at establishing the net irrigation requirement of cabbage crops (Zavadil, J., 2006; Ayas, S., 2019). Yordanova, M., 2013 made an experiment with cabbage, testing three different rate of drip irrigation. The author found that the cultivation of cabbage for late field production, with a reduction in the irrigation rate (60% m), leads to a decrease in yields of 4-16%, depending on the weather conditions. Increasing the irrigation rate above the optimum (120% m) does not lead to a directly proportional increase in yields but has a depressing effect and lowers yields by 9 to 18%.

Leskovar D.I. (2014) based on the field experiment made conclusion that deficit irrigation at 75% ETc had little influence on plant size, leaf pigment content, leaf characteristics, head weight and size, except for a moderate reduction in marketable yield.

The aim of study is to establish the influence of different irrigation regimes on influence of deficit irrigation on cabbage growth, head weight, size and yield for the conditions of the Sofia region.

MATERIALS AND METHODS

The experiment was carried out during the 2022 in the area of the Vrajdebna-Sofia. The soil type is alluvial-meadow. The standard for the Bulgaria variety of cabbage "Kyose" is used. The experiment was carried out using the method of long plots in four replications, with the size of the harvest plots - 12 m^2 and the sowing scheme being a two-row strip with an inter-row distance of 70 x 50 cm.

To evaluate the influence of the irrigation regime on growth, development, and yield the vegetation period of cabbage is divided into two sub-periods, namely: "vegetative" and "reproductive".

Different variants of the irrigation regime were tested as follows:

Var. 1. Irrigation with 40% of the irrigation rate determined in the optimal variant.

Var. 2. Irrigation with 70% of the irrigation rate determined in the optimal variant.

Var. 3. Irrigation with full irrigation rate (100% m) - optimal irrigation (control).

Var. 4. Irrigation with an increased irrigation rate (130% m) - determined in the optimal variant.

Var. 5. Without irrigation during the "planting-vegetative growth" period.

Var. 6. Without irrigation during the "head formatting" period.

Irrigation in the optimal variant (var. 3) will be applied when the soil moisture drops to 80% of the field capacity (FC) in the 0-40 cm layer, and the size of the irrigation rate will be determined to moisten the entire active soil layer (0-60 cm). For this purpose, the dynamics of soil moisture will be monitored during 5-7 days using the Gravimetric Soil Moisture Detection (S.G. Reynolds, 1970).

GWC (%) = [(mass of moist soil (g) − mass of dry soil (g)) / mass of dry soil (g)] × 100

The irrigation rate in the optimal variant will be calculated according to a formula (G. Krafti et al., 1969; Z. Stoyanov et al., 1981; G. Georgiev et al., 1991), based on the water balance equation.

The experimental plots will be irrigated with drip hoses, in order to precisely dose the irrigation water. The amount of water delivered to each of the irrigated plots will be measured on an hourly flow rate basis.

Biometric measurements were performed on 10 plants from each plot in four replicates. The diameter and height of the head, average weight per head and yield.

Water content in cabbage head was measuring using the formula (Jin X. et al., 2017):

Water content (%) = (Wf−Wd)/Wf∗100

Where: Wf - fresh weight and

Wd - dry weight.

The amount of precipitation is recorded with a rain gauge in the morning. No fertilization during the cabbage growth was applied.

RESULTS AND DISCUSSIONS

During the growing season, average daily air temperatures and the amount of precipitation were recorded (Figure 1). The annual amount of precipitation is 613.8 mm, which defines the year as average in terms of precipitation. However, the precipitation during the growing season of cabbage is 269.6 mm, which leads to a good supply of soil moisture, which is around 80-90% of the maximum field moisture capacity for this soil type (21.1 FC).

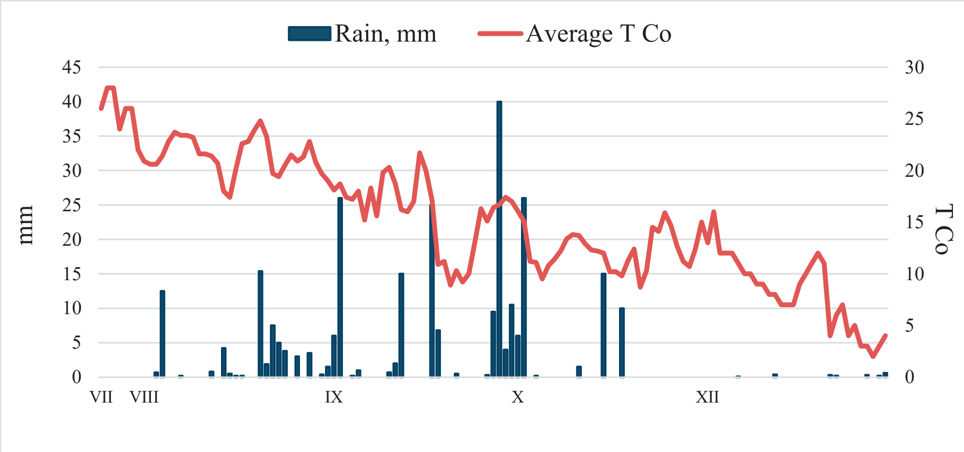

Figure 1. The precipitation and average air temperatures for 2022, filed experiment Vrajdebna

An even distribution of precipitation is observed during the vegetative phase of cabbage development. This is the reason for realizing a smaller number of irrigations. In the month of September, two large rainfalls of 26 and 40 mm were recorded. On 28.09. 2022 y. along with the 40 mm of rain, there was hail the size of a walnut, making it impossible to measure the diameter of the rosette. The large amount of precipitation leads to a reduction in the effect of the applied irrigation regimes. This is also the main reason for obtaining the highest yields in variant 6 (without irrigation during the "head formatting" period).

The average daily temperature ranges between 28°C at the end of June and 2°C in November. In mid-September, a sharp drop in temperature was observed, dropping from 21.7°C to 8.9°C.

Cabbage was harvested at the beginning of November. The same trend is observed for all reported indicators for the quality of the harvest, the lowest indicators are reported for variant 3 which is optimal, and variant 4 with the increased irrigation rate. This is due to waterlogging of the soil profile, which leads to stress and difficult work for the root system. Yordanova M. (2013) reported a reduction in yield by 9-10% at an irrigation rate of 120% of the optimal rate.

The smallest head height of 17 cm was recorded for variants 3 (100% of the irrigation rate) and var. 4 (130%) of the irrigation rate. In the variant with a reduced irrigation rate, var. 1 (40%) has a height of 19.2 cm, while the highest result is for variant 6 with 19.7 cm. Values in a column are significantly different at $P \leq 0.05$. The statistical analysis of the data showed very good evidence of differences between variants (P=1.08170542218746E-06). The diameter of the head follows the same tendency. Again, the smallest width was reported for variants 3 and 4 (19.4 cm and 18.99 cm), the removal of the irrigation during the second phase of development also had a favourable effect on the width of the head (21.22 cm) (Figure 2).

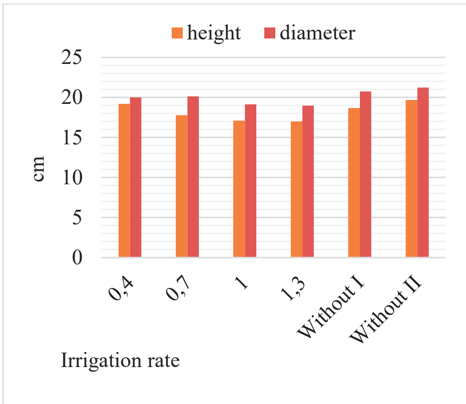

Figure 2. Effect of irrigation rate on the head size of cabbage sp. Kyose 2022

The reported fresh weight of the heads keeps the same trend. The values for variants 3 and 4 are very close and are the lowest for the variants (1.55 kg). The weight of the cabbage heads in variant 6 is 15% greater than that reported in the flooded variant (2.33 kg). The difference between the variants with 1 (40%) and 2 (70%) is minimal, the values are very close for the parameters of the product part. The results of the Anova statistical program show (P= 7.98331E-06) (Figure 3).

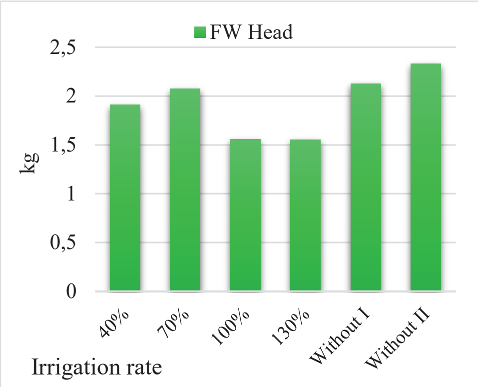

Figure 3. Effect of irrigation rate on the head weight of cabbage sp. Kyose 2022

The best results in the development of the product part of the cabbage variety Kyose are reported in the variant with cancellation of irrigation during the "head formation" period (var.6).

The obtained results for the parameters of quality and quantity of production clearly show the negative impact of waterlogging on white cabbage. In confirmation of this, there are also data obtained from other authors. In studies done on the response of white cabbage to waterlogging, Huđ A, at al 2023, reported that in short-term waterlogging of cabbage, the plants showed signs of stress. The reported data show that plants exposed to a single waterlogging event in the early growth stage were not stressed. While repeated waterlogging in the later stages of development induced a metabolic response in the plants. Casierra-Posada and Cutler 2017 demonstrated that prolonged waterlogging significantly reduced leaf area, total dry weight, chlorophyll content, leaf area ratio, absolute growth rate, and relative plant growth rate.

To determine the absolute dry mass of the productive part of the cabbage, an average sample of 3 heads weighing 300g was taken. The absolute dry mass varies from 24.57 g to 29.6 g. The driest mass is accumulated in var.4 followed by var. 6 (Figure 4).

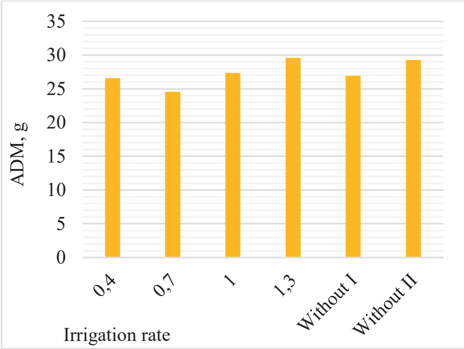

Figure 4. Effect of irrigation rate on the absolute dry mater of cabbage sp. Kyose 2022

The moisture contents of the cabbage samples were opposite those obtained for the dry mass. The highest moisture content is option 2 (70% of the irrigation rate) followed by var.1. The lowest values are for var. 4 followed by var. 6 (Figure 5).

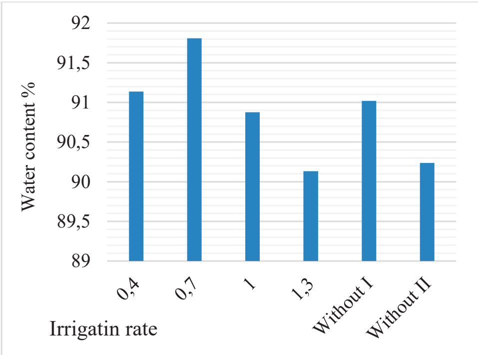

Figure 5. Effect of irrigation rate on the water contend in marketable part of cabbage sp. Kyose 2022

The yield was calculated based on a planting density of 2,857 plants per ha^{-1}. The lowest yields were reported for var. 3 (44.5 t/ha) and var. 4 (44.41 t/ha). The yield obtained in the variants with skipped irrigation is 66.4 t/h (Figure 6).

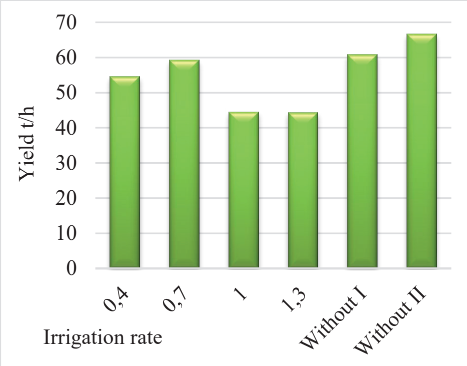

Figure 6. Effect of irrigation rate on the yield in marketable part of cabbage sp. Kyose 2022

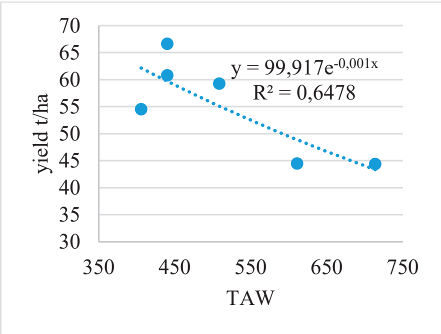

Figure 7. Dependence yield amount of available water

The Figure 7 clearly shows the dependence of the increase in yield on the increase for water. After soil moisture exceeded the level by 500 mm, a decrease in yield was observed. At a humidity level of 713 mm, the yield drops sharply. The negative influence of soil waterlogging on yield is clearly outlined. The applied irrigation rates and subsequent rainfall far exceed the net irrigation requirement for cabbage. According to data from the regulation on water consumption of the Republic of Bulgaria from December 22, 2016, the net irrigation requirement varies from 310 to 520 mm, depending on the year's climate characteristics.

CONCLUSIONS

Based on the data obtained from the conducted experiment, the negative influence of waterlogging on the size, weight, and yield of late cabbage is clearly outlined. The cancellation of irrigation in the phase of head thickening, combined with the fallen precipitation, leads to obtaining the highest yields of 66.4 t/h.

Cultivation of cabbage with a disturbed irrigation regime is a promising way to reduce the irrigation rate and increase water use efficiency.

Based on the obtained results, we can recommend reducing the irrigation rate by 70%, in yields of 59.25 t/ha. Applying a disturbed irrigation regime leads to water savings with a slight decrease in yield.

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PLANTING TIME EFFECT ON THE GROWTH AND YIELD OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

Nikolina SHOPOVA

Agricultural University-Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: nina_sm@abv.bg

Abstract

The aim of the experiment was to investigate the effect of planting time on the growth and yield of tomatoes, grown in conditions of late field production. The experiments were carried out during the period 2020-2021 in the experimental field, Department of Horticulture at Agricultural University - Plovdiv, with cultivar Opal F_1. The opportunity of growing and fruiting of plants was evaluated by planting on three dates through 15 days (20th June, 5th, and 20th July). During the growing season, plant height (cm), leaf area (cm^2), first flowering and first fruiting, fruit set, and yield (kg/da), were recorded. The planting time has effects on the growth and yield of tomatoes. The better performance of tomato plants was observed planting on the 20th of June as the plants fall into climatic conditions near optimal for the crop, which affects the vegetative and generative growth of plants and increases the total yield.

Key words: *tomato, planting time, growth, yield.*

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is one of the economically significant vegetable crops, both globally and in Bulgaria (Meza et al., 2013; MZH, 2021). Tomato fruits contain macro- and micronutrients, antioxidants, vitamins A and C, which greatly support human health, which is why their consumption is increasing worldwide (Adalid et al., 2004; Luthria et al., 2006; Nour et al., 2013). In Bulgaria, suitable atmospheric conditions for tomato production are from April to October. Tomatoes are grown in three directions - early, mid-early, and late field production (Shaban et al., 2014). According to the adopting technology for growing late tomatoes, the seeds are sowed during the period June 1-5 and transplanted on 1-5 July (Cholakov, 2009). The production of late field tomatoes takes place in the period July - October, and part of the production is realized through post-harvest ripening of the fruits (Cholakov, 1987; Shopova & Cholakov 2013).

According to Cholakov (2009), the high requirements for the variety for the production of late-field tomatoes are determined by the climatic conditions during the growing season. The vegetative and generative development of plants takes place in the conditions of higher summer temperatures (July and August), and fruiting in September-October. In this period, the difference between day and night temperature increases, which negatively affects the plants, creates conditions for the development of diseases and the risk of fruit cracking. In this regard, air conditions during the growing season are particularly important for development and fruiting (Popov et al., 2003). As is known, tomatoes are grown in a wide range of climatic conditions but are sensitive to high temperatures (Alam et al., 2010).

The appropriate planting date is a major factor in vegetable production, directly related to the climatic conditions of the respective area (Rahman et al., 2020). Different planting days can affect the yield and quality of the production due to changing climatic conditions, and different stages of growth and development of the crop (Gent, 1992; Hossain, 2021). According to Emami (2014) determining a suitable date for transplanting tomatoes, is important to obtain early harvest and a high yield of tomatoes. According to Cebert et al. (1990), planting dates for one cultivar may not be suitable for another. For some plant species, different planting dates will affect only vegetative growth but will have no effect on reproductive growth.

The purpose of the conducted research was to determine the change in the growth and development of late tomatoes depending on the planting period.

MATERIALS AND METHODS

The experimental work was conducted in the period 2020-2021 in the Department of Horticulture, Agricultural University of Plovdiv, Bulgaria with tomatoes variety Opal F_1. Sowing of seeds was carried out during the period May 20, June 5, and June 20. The seedlings were grown in trays with 77 cells. The planting was carried out during the period - June 20 (first date), July 5 (second date), and July 20 (third date). Thirty-day seedlings were used for all three planting dates. The betting of the experiment was done in three repetitions in the high bed-furrow surface by a schema - 100 + 60/40 cm. The plants were grown under conditions of drip irrigation. The cultivation of the plants was carried out according to the adopted technology for late field production with the attachment of the plants to the structure, regular pruning, and single-stem formation, with the removal of the stem after the formation of 4th inflorescence (Cholakov, 2009). During the growing season, a biometric measurement was performed on the 40th day after planting on each study date.

Experiment 2020

Experiment 2021

The data on the phenological development of plants like days to first flowering, first fruiting, first fruit maturity, and days to the end of the harvest period were recorded. The numbers of flowers per plant, fruits per plant, and fruiting set (i.s. the percentage ratio between the number of flowers and fruits) were determined. The yield was determined by month and total for the period.

RESULTS AND DISCUSSIONS

The air temperature is the main meteorological factor affecting plant development (Kalbarczyk et al., 2011). The average daily temperatures (Table 1) for July, August, and September, during which most of the vegetation of plants takes place, were within the limits of the optimal range for the development of the plants. The optimum growth temperature of tomatoes is within a range of 18.3 and 32.2°C (Hochmuth & Hochmuth, 2012). The average daily temperature in June, when the plants from the first investigation date were planted, was also within the optimum range. The average maximum temperature for June, July, August, and September was close to the developmental maximum. According to Kittas et al., (2005), the growth of tomato plants stops below 10ºC and above 35ºC. The average minimum temperatures, except for the ones reported in October, were above the agro biological minimum (10ºC). In connection with the lowering of temperatures in October, it is worth noting that in both experimental years until the end of this month, no autumn frost was formed. An important meteorological element that is directly related to the pollination and fertilization processes is relative humidity. The most favorable for plants is the moderate relative humidity between 60-70% (Huang et al., 2011). In July and August, when most of the fruits of late tomatoes are formed, the reported values were 62% in 2020 and 57-55% in 2021, respectively. The relative humidity in September is important for the third experimental date, which averages 57% for 2020 and 62% for 2021. In conclusion, it can be noted that the climatic conditions during the two experimental years were relatively favorable for growing late tomatoes.

Table 1. Climatic characteristics of the area

Meteorological parameters	June		July		August		September		October	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Average daily temperature	21,4	23,4	23,5	24,0	24,6	24,8	21,3	19,9	15,2	14,3
Average max. temperature	27,7	29,6	31,1	31,8	32,2	32,3	28,9	29,3	22,3	20,1
Average min. temperature	15,5	17,2	16,7	16,9	16,3	17,4	13,4	12,0	9,2	6,1
Absolute max. temperature	34,0	34,7	34,2	37,2	35,6	37,0	35,0	34,5	28,5	27,5
Absolute min. temperature	11,3	10,6	12,0	13,2	13,3	12,4	2,8	4,8	1,7	1,3
Relative humidity %	67,0	66,4	62,0	57,0	62,0	55,0	57,0	62,0	74,0	78,0
Rainfall, mm	55,4	45,4	20,0	10,6	17,8	49,7	1,3	9,2	62,3	110,4

Table 2. Phenological phenophases of development of the plant

Planting date on:	DURATION IN DAYS TO:							
	first flowering		first fruiting		first maturity/ first harvest		last harvest	
	2020	2021	2020	2021	2020	2021	2020	2021
20 June	40	41	47	48	85	86	138	136
5 July	40	41	48	49	87	88	145	144
20 July	44	45	52	54	94	95	131	135

The course of phenological phases has an important role in yield quantity and quality (Mozny et al, 2009; Tao et al, 2006; Vlahova, 2012; Panayotov et al., 2020). The occurrence of phenophases is weakly affected by the planting period (Table 2). On the first and second dates of planting, the flowering of the first inflorescence occurs on average 40-41 days after germination, while on the third date after 44-45 days. This delay is most likely due to that after the 2rd decade of July, the plants fall under conditions of higher temperatures. For all three planting dates, a longer period until the flowering was reported in 2021. A similar trend was observed for the phenophases of first fruiting and maturity. For all three dates, the period of reddening of the first fruit coincides with the first harvest. Traditionally, for late field tomato production, the last harvest is done before the first autumn frost (Cholakov, 1987). In the second planting time, the duration of the harvesting period is 58 days for 2020 and 56 days for 2021. The duration of the harvest period for the June planting was 53 days for 2020 and 50 days for 2021, and the last harvests were made in early October. At the third tested date, the harvest period is shortest - 37 days in 2020 and 40 days in 2021.

The results of the biometric measurements performed on the 40th day after planting (Table 3) showed a weak influence of the planting period on the main biometric parameters.

Despite the similar values of the reported biometric indicators, it should be noted that the plants with earlier planting have the largest stem-leaf mass, and the plants from the third date are the least. The differences between the investigated variants are most likely due to the different climatic conditions during the early stages after planting the plants. June planting coincides with moderately high average daily temperatures, allowing for faster plant establishment and more intense plant growth in the first few days after planting before the sustained increase in summer temperatures occurs in July. The plant's height is 110.7 cm, the number of leaves per plant was 24.2, the leaf area - was 4697.5 cm^2, and stem-leaf mass was the least developed.

Table 3. Biometric measurement per plant on the 40th day after planting

Planting date on:	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Stem-leaf mass (g)
2020				
20 June	116,1 a	23,8	4824 a	705
5 July	110,0 a	20,0	4588 ab	671
20 July	107,2 a	21,2	4080 b	619
2021				
20 June	105,3 a	24,6	4571 a	586
5 July	104,0 a	22,8	4178 b	521
20 July	98,2 a	22,0	4008 b	486
Average for the period				
20 June	110,7 a	24,2	4697,5 a	645,5
5 July	107,0 a	21,4	4383,0 ab	596,0
20 July	102,6 a	21,6	4044,0 b	552,5

Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

In all variants, the vegetative peak of the plants was removed after the formation of the fourth inflorescence. The total number of flowers and fruits per plant was obtained as the sum of the flowers and fruits formed in each inflorescence. This makes it possible to determine the fruit set per plant (Table 4). The number of flowers and the degree of fruit set was directly related to the size of the yield.

Depending on the date of planting in 2020 the number of flowers was 21.3-24.7 and the number of fruits per plant was 18.4-22.6 (a fruiting rate of 86.4-91.5%). In the second experimental year, the number of flowers was 20.2-23.8 per plant, and the number of fruits was 17.4-21.8. The reported fruit set in 2020 was higher, which shows that the conditions for pollination, fertilization, and fruiting were more favorable. As it knows, tomatoes are grown under a wide range of climatic conditions but are sensitive to hot and humid environments (Alam, 2010). High air temperature reduces the fertility rate of flowers, resulting in flower drop and fruit set. Tomato plants drop flowers when exposed to several days of daytime temperatures above 29°C and nighttime temperatures above 21°C (Ayankojo & Morgan, 2020). Values higher than 35°C will also reduce fruit set and delay the development of normal fruit colors (Jones, 2013).

Table 4. Number of flowers and fruits per plant, and fruit set (%)

Date of planting	2020			2021			Average		
	Flower per plant	Fruit pre plant	Fruit set %	Flower per plant	Fruit pre plant	Fruit set %	Flower per plant	Fruit pre plant	Fruit set %
20 June	24,7	22,6	91,5	23,8	21,8	91,6	24,3	22,2	91,5
5 July	22,4	20,0	89,3	21,8	19,4	89,0	22,1	19,7	89,1
20 July	21,3	18,4	86,4	20,2	17,4	86,1	20,8	17,9	86,3

On average for the two-year period, the generative potential of plants was 20.8-24.3 flowers, the number of fruits was 17.9-22.2, fruit set of 86.3-91.5%. The number of flowers and fruits per plant and the fruit set was highest in June planting.

The fruit yield shows most objectively the influence of the studied factor on plant productivity. In the three variants, the values of this indicator were higher in 2020 (Table 5). The difference in yield between the first and second date of planting was small - 258.3 kg in 2020 and 334.2 kg in 2021 and differences have not been proven.

A higher yield was reported in June planting, and it decreased with later planting. The higher yield corresponds to the results for the flowering and fruiting of plants in this variant. The yield from the third date was the lowest and the differences are statistically proven. The distribution of yield by months is important for the economic efficiency of production. In 2020, in August, 6.1% of the total yield is obtained in planting in early July and 45.2% in planting in June. In September, 64% of the total yield was harvested (5 July), and from 46.3% to 48.5% in the other two investigated planting dates.

Table 5. Effect of planting time on yield kg/da

Planting date on:	2020	2021	average	Distribution of yield by month, average for the period						
	kg/da	kg/da	kg/da	August kg/da	% to Total yield	September kg/da	% to Total yield	October		% to Total yield
								Total yield kg/da	The yield of green for post-harvest kg/da	
20June	5768,7 a	5656,2 a	5712,5 a	2582,0	45,2	2770,5	48,5	359,9	0,0	6,3
5 July	5510,4ab	5322,0 ab	5416,2 a	330,9	6,1	3466,4	64,0	1619,4	478	29,9
20 July	5056,2 b	4856,3 b	4956,3 b	0,0	0,0	2294,8	46,3	2661,5	1025,1	53,7

Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

The amount of harvested fruit was the highest in September.

In October, the yield was highest in late July planting (20 July). Warmer and longer autumn favors the ripening of most fruits. At the end of the harvesting period, 2661.5 kg/da was harvested, of which 1025.1 kg/da was for post-harvest ripening.

During early July planting, the yield was 1619.4 kg/da, of which 478 kg/da was for post-harvest ripening. At the June planting, the yield of 359,9 kg was formed only from ripe fruits, as the harvesting period ends in the first ten days of October.

CONCLUSIONS

The experiment showed that the vegetative and generative development of late tomatoes was influenced by the date of planting. Planting on June 20 increases the number of flowers and fruits, fruit set, and the total yield. Depending on the date of planting, in August was obtained from 6.1-45.2% of the total yield, from 48.5-64% - in September, and from 6.3-53.7% - in October. When the purpose of production is to extend the consumption period of fresh fruit by post-harvest ripening, July planting is appropriate.

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DIVERSITY OF COMMON BEAN LANDRACES (*PHASEOLUS* SPP.) MAINTAINED IN HOME GARDENS IN BULGARIA

Tsvetelina STOILOVA, Petar CHAVDAROV

Institute of Plant Genetic Resources, 2 Drugba Str., Sadovo, Bulgaria

Corresponding author email: tz_st@abv.bg

Abstract

Dry bean is considered as one of the major legume crop with big number of landraces grown in Bulgaria. The aim of this study was to characterize landraces of Phaseolus spp. typically grown in home gardens. Studied plant materials were selected according to their importance and distribution, concerning traditions and culture for each region. The most popular local forms were characterized by different approaches. In this paper we will present results from morphological and agro-biological characterization, and phythopathological evaluation. During vegetation cycle of three consequences years 16 qualitative and quantitative traits were assessed on 55 accessions of Phaseolus spp. Phaseolus landraces showed high seed diversity, in terms of seed size, seed shape and seed color. Seed size was analyzed by 100 seed weight. Most of the studied accessions were scored with sensitive reaction to bacterial diseases (Xanthomonas campestris pv. Phaseoli) and only few accessions were identified with middle sensitive reaction.

Key words*: characterization, common bean, diversity, evaluation, local origin.*

INTRODUCTION

Grain legumes provide high quality proteins for many people of the world. Among the pulse crops dry beans (*Phaseolus vulgaris* L.) are the second most important legume crop after soybean. Dry beans are an important staple food in many cultures around the world; it is one of the basic food in Latin America, India and Africa (Seidel, 2022). A big genetic and phenotypic diversity was observed in many countries where it is grown in a wide range of environments to which it is adapted (De Ron et al., 2016). Dry beans are grown in almost all European member countries whereas Spain is one of the main producer and consumer (CBI, 2022). Savic et al. (2019, 2021) studied phenotypic and microsatellite markers diversity of Serbian collection of common beans and proved to be polymorphic, whereas landraces displayed higher variability compared to cultivars included in this study. Pipan & Meglic (2019) conducted a study to define level of diversification of common bean (*Ph. vulgaris* L.) from western to eastern line of Southern European countries, from Portugal to Ukraine. The authors concluded that the collection of 782 accessions represented a valuable source of genetic variability. In Brasil seventeen common bean genotypes were examined on genetic diversity of agronomic traits, the authors selected superior genotypes and defined promising crosses (Ribeiro et al., 2022). In Bulgaria dry beans is considered as one of the major traditional legume crop with big number of landraces grown all over the country by both commercial and small scale farmers (Stoilova, 2013). Landraces are developed by farmers over many generations and are well adapted to specific climatic conditions. They are plants with high ecological plasticity, with good behaviour to abiotic and biotic stress factors. Most farmers prefer old populations and primitive varieties hence the large diversity of these populations and excellent organoleptic taste. Farmers still keep their seeds and grown in home gardens in rural and marginal areas (Savic et al., 2021). They are traditionally grown under low input farming system, adapted to local agro-climatic conditions and display high level of phenotypic diversity (Carovic et al., 2017). Nevertheless, there is a risk of extinctions of many landraces due to aged farmers, new bred cultivars and the socio-cultural context where they are maintained (Mallor et al., 2018) The national bean collection is preserved at the National Genebank which belongs to the Institute of Plant Genetic Resources (IPGR), Sadovo. The collection consists at about 2200 accessions, with local and foreign origin (Stoilova et al., 2013). The big number of landraces collected during several decades provide opportunity to select the most suitable of them for the respective breeding objectives or to be repatriated to the farmers for direct use, in case of farmer's interest. On recent years, several bean populations and landraces mostly belonging to *Ph. vulgaris* and *Ph. coccineus* were collected, characterized and evaluated (Stoilova, 2011). Characterization of genetic resources remained the first step to investigate the level of variation of morphological, agro-biological and agronomic traits which can be used for crop improvement in terms of high yield potential with better quality of production, very good organoleptic taste, biotic and abiotic stress tolerance. Characterization and evaluation are of main importance for conservation plant genetic material *ex situ* in genebank or *in situ*/on farm in their origin in view of future breeding and research work under global climate change. The aim of our study was to make a complex morphological, agro-biological and agronomic characteristics of the 55 accessions, landraces with local origin in view of their better utilization and conservation.

MATERIALS AND METHODS

The study took place from 2020 to 2022 at the experimental field of IPGR, Sadovo (49◦9´N, 24◦57´S and 158m a.s.). The study was carried out on 55 common bean (*Ph. vulgaris* L.) accessions with local origin from different regions of the country (Figure 1).
Sowing took place in first half of April depending from the meteorological conditions during the certan period. The field trial is sown on cinnamon-forest soil and usually follow the predecessor cereals. All accessions were sown in three replications on an experimental plot of 5.6 m^2. Basic phenological stages, morphological and agronomic traits were recorded during the vegetation period, using Bioversity International Phaseolus descriptor (1982). Ten plants from each accession per replication were taken for biometric measurements. The phenological observations were done on number (nr) of days to reach 50% flowering, duration of flowering, nr of days to maturity (95% matured plants), while the vegetative and reproductive organs were measured during full developed height of plants, leaves, pods and seeds. Observations on resistance to two bacterial diseases, caused by the pathogens, *Xanthomonas axonopodis* pv. *phaseoli* and *Pseudomonas syringae pv. phaseolicola* were made using the score 1-9, based on method of Genchev & Kiryakov (2005) and Bioversity Int. (1982).

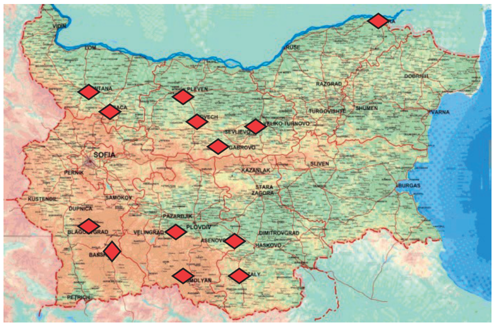

Figure 1. Origin of studied accessions.

Cluster analysis and Principal Component Analysis (PCA) were performed by UPGMA method on 19 quantitative and qualitative traits using SPSS statistical programme.

RESULTS AND DISCUSSIONS

The accessions included in this study needed 59.1 days (mean value) to reach 50% flowering (Figure 2). Four accessions, cat. No 811355, cat. No 741356, cat. No 761376, cat. No 62258 showed the longest period to reach flowering and maturity stages with 69 and 98 days, respectively. Two accessions, cat. No A7E0668 and cat. No B9E0001 started flowering earlier, after 53 days, compared with all other accessions they reach maturity for shorter period of 81 days. Duration of flowering (DurFl) ranged from 30 to 40 days, the shortest flowering period of 30 days was registered in 54.5%, the longest period of 40 days flowering was observed in 31% and rest of accessions 14.5% were with mean number of days - 33-35 (Figure 2).

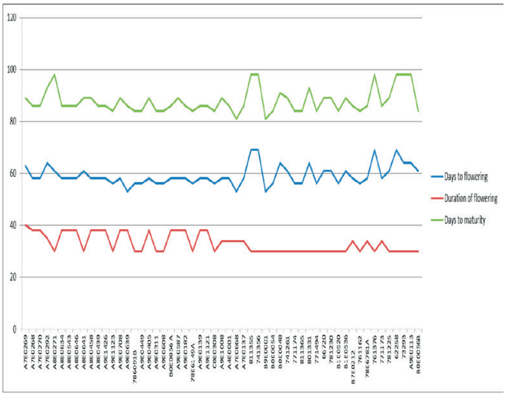

Figure 2. Phenological stages of 55 accessions

Coefficient of variation of the three phenological characters showed low values, as follows: DF-6.6%, DurFl-11.1% and DM - 5.3%, respectively (Table 1). Duration of flowering differs by growth habit of plants, where accessions with indeterminate type flowered and matured for longer cycle compared with accessions possessing determinate growth habit.

Table 1. Descriptive statistics of phenological characters of common beans

Statistical values	Days to 50% flowering (DF)	Duration of flowering (Dur.Fl.)	Days to 95% maturity (DM)
Minimum	53	30	81
Maximum	69	40	98
Mean	59,1	33,1	87,8
Standard Error	0,5	0,5	0,6
CV (%)	**6,60**	**11,10**	**5,30**

Morphological traits (h of plant, weight of pl., number of branches, weight of pl./without pods, h of 1st pod, number of pods/pl., weight of pods/pl., pod length, pod width and pod thickness. Plant growth habit of studied accessions belonged to determinate /erect type - 61.8% and indeterminate/ climbing type 38.2%. Consequently, morphological traits, as follows: h of plant, weight of plant and weight of plant/without pods were with highest value of coefficient of variation (Table 2). Accessions with cat. No A8E0641, A9E0311, A9E0182, 771174, 801331, B7E0212, 761376, 73293 were with the biggest plant weight (weight of plant without pods-WPWP) > 30 g, at the same time 25 accessions showed less than 10 g WPWP. Twenty two accessions were between 10 and 30 g. It needs to be noticed plant growth habit was not connected with weight of plant and weight of plant without pods. Some of the accessions with indeterminate type didn't reveal their potential growth habit under agro-climatic conditions of Sadovo. High T◦C and low relative humidity (RH%) during flowering and beginning of podding stages which caused heat stress resulted in abortion of flowers and young pods, leaded to low yield production. Similar results were reported by Da Silva et al. (2020) and Vargas et al. (2021). Number of pods and weight of pods per plant showed high value of CV (%) - 44.09 and 44.62%. Different genotypes produced different number of pods, with range from 3.6 to 20 with mean value of 8.2 pods/pl. and CV (%) - 44.1. Accession with cat. No 741261 produced the biggest number of pods (20) and three accessions (A9E0608, B9E0001 and 781230) >15 pods/plant. All mentioned accessions had erect growth habit and were comparatively early maturity (81-84 DM). Pod length ranged between 6.7 to 14.1 with mean value of 9.5 cm and variation among all accession was the lowest with CV-15.8%

Table 2. Morphological traits of 55 accessions of common bean

Cat.№	h of plant (cm)	Weight of plant (g)	Number of branches	Weight of plant/without pods (g)
A7E0269	33,24	18,4	2	8,2
A7E0268	24,8	15,3	2	7,6
A7E0270	29	9,4	3	3,5
A7E0292	34,2	13,0	2	7,0
A8E0271	107,2	17,0	3,2	11
A8E0634	113	38,5	3,6	29,1
A8E0543	106,8	8,9	3	4,9
A8E0646	31,8	33,5	3,8	17,9
A8E0641	100,2	51,1	3	34,1
A8E0458	39	21,4	3	15,2
A8E0439	78,6	22,2	3,1	14,0
A9E1426	34,8	9,3	2,6	3,9
A9E1123	80	22,4	3	15,5
A9E0708	107,6	12,1	3	9,5
A9E0639	30,8	7,1	2	3,6
786091B	39	15,3	2	5,2

Cat.№	h of plant (cm)	Weight of plant (g)	Number of branches	Weight of plant/without pods (g)
A9E0449	78,4	21,3	3	9,1
A9E0405	65,4	13,5	2,2	6,4
A9E0311	96,3	53,2	2,3	44,6
A9E0608	32,2	41,8	2,2	18,1
B0E0056 A	39,6	22,5	2,2	8,9
A9E0587	100,6	24,5	3	9,8
A9E0182	67,8	64,1	3	40,0
78E6149A	34,8	13,6	2	6,0
A9E0139	37,2	20,8	2,2	12,7
A9E1121	36,4	28,9	2	12,6
C0E0308	111,2	18,8	3	12
A9E1008	33,2	19,9	2,2	11,4
A4E0001	33,2	14,3	2,2	5,8
A7E0668	37,6	14,9	2	4,9
A7E0137	41	9,7	3	5,0
811355	54,2	15,6	2,4	9,3
741356	105	30,8	3	16,9
B9E0001	37,4	34,5	3	19,4
B0E0054	39	15,8	2,4	4,1
B0E0048	41,6	32,3	2,2	22,3
741261	40,8	33,5	2,8	15,5
771174	38,8	37,1	2,8	31,9
811365	35	15,5	2	9,5
801331	41	44,8	3	30,4
771494	40,8	31,6	2,2	10,0
66720	33,4	22,4	2	5,2
781230	38,4	33,7	2,8	18,2
B1E0520	38,2	24,6	2,6	13,2
B1E0536	27	18,4	2,4	10,3
B7E0212	125,2	46,2	4	31,8
761162	29,8	12,2	2	3,8
78E6781A	33,6	29,8	2,2	11,8
761376	106	49,5	3	39,0
771173	109,6	22,3	3,2	15,9
781225	103,2	20,2	3,4	6,8
62258	38,2	21,4	2	10,5
73293	111,6	58,0	4	38,7
A9E0113	66,4	18,8	3	9,8
B0E0056B	37,8	15,9	2	10,0
Minimum	24,8	7,1	2	3,5
Maximum	125,2	64,1	4	44,6
Mean	58,3	25,1	2,6	14,4
StE	4,3	1,8	0,1	1,4
CV, %	54,18	53,6	21,2	73,7

Table 2. Morphological traits of 55 accessions of common bean - Continued

Cat.№	h 1st pod (cm)	Nr of pods/pl	Weight of pods/pl (g)	Pod length (cm)	Pod width (mm)	Pod tickness (mm)
A7E0269	13,4	7,8	9,9	10,5	1,0	0,8
A7E0268	10	4,8	7,1	11,4	1,2	0,6
A7E0270	13,4	5,2	5,7	9,1	0,9	0,8
A7E0292	11,8	4,4	5,7	10,5	0,7	0,6
A8E0271	19,6	4,4	5,5	8,7	0,8	0,6
A8E0634	10,2	5,6	8,5	10,7	1,2	1,0
A8E0543	10,8	4,4	3,9	7,7	0,9	0,6
A8E0646	9,6	9,4	15,3	10,1	1,2	0,7
A8E0641	12,4	12	16,1	7,9	0,9	0,7
A8E0458	12,2	6	5,8	7,5	1,0	0,7
A8E0439	12,4	5,6	7,8	9,5	0,9	0,7
A9E1426	9,4	5,6	5,2	7,6	0,8	0,5
A9E1123	12	4	6,8	9,8	1	0,6
A9E0708	14,8	4,6	4,1	7,8	0,8	0,7
A9E0639	7,8	3,6	3,4	6,7	0,7	0,6
786091B	9	7	9,8	9,8	0,9	0,8
A9E0449	11,4	9	11,9	9,7	1,0	0,6
A9E0405	10	7,2	8,8	9,5	0,9	0,8
A9E0311	11,0	5,7	8,1	11,4	0,8	0,5
A9E0608	8,2	15,6	22,6	9,6	0,8	0,7
B0E0056 A	10,6	8	12,8	10,9	0,9	0,7
A9E0587	14	8,4	14,1	10,5	0,9	0,8
A9E0182	20,4	10,8	23,4	11,8	1,4	0,8
78E6149A	8,2	4,4	6,9	11,4	0,8	0,9
A9E0139	9,2	6	7,5	10,7	0,8	0,8
A9E1121	10	7,8	15,9	8,9	1,2	0,9
C0E0308	12,4	9,8	6,8	9,5	0,7	0,5
A9E1008	16,6	6	8,1	11,5	0,9	0,7
A4E0001	9,2	5	7,7	11,0	0,9	0,5
A7E0668	12	8,2	9,2	9,5	0,9	0,5
A7E0137	17	4,2	4,2	9,0	1,0	0,6
811355	14	7	5,9	9,8	0,6	0,6
741356	9	13,4	13,4	8,1	0,7	0,6
B9E0001	18,4	15,2	14,6	8,7	0,6	0,5
B0E0054	15,2	5,8	11,2	10,6	1,4	0,8
B0E0048	9,2	8,2	9,3	8,7	0,6	0,6
741261	13	20,0	17,3	7,4	0,7	0,6
771174	10,8	3,6	5	10,0	0,7	0,6
811365	18,6	7,2	5,9	9,5	0,7	0,6
801331	8,8	14,4	14,0	14,1	0,9	0,6
771494	10,2	10,8	16,9	11,6	1,1	0,8
66720	7,8	10,2	16,7	11,8	0,9	0,6
781230	9,8	15,6	15,2	9,8	0,8	0,6
B1E0520	11,8	8,2	10,8	9,8	1,1	0,8

Cat.№	h 1st pod (cm)	Nr of pods/pl	Weight of pods/pl (g)	Pod length (cm)	Pod width (mm)	Pod tickness (mm)
B1E0536	6,6	6,8	7,9	9,9	0,7	0,6
B7E0212	14,2	8,4	13,8	9,9	1,1	0,9
761162	7,6	8,4	8,0	7,0	0,8	0,5
78E6781A	10	12	15,0	10,7	0,8	0,5
761376	7,8	11,8	9,8	6,9	1,1	0,7
771173	8,8	6,8	6,1	9,1	0,9	0,6
781225	13	9,8	12,8	8,0	1,1	0,8
62258	9,6	10,8	10,0	8,0	0,8	0,5
73293	10,2	12,8	17,6	8,0	1,8	0,5
A9E0113	11,6	8,2	8,2	8,9	1,0	0,7
B0E0056B	8,2	4,6	5,5	7,7	0,8	0,7
Minimum	6,6	3,6	3,4	6,7	0,6	0,5
Maximum	20,4	20	23,4	14,1	1,8	1,0
Mean	11,5	8,2	10,2	9,5	0,9	0,7
StE	0,4	0,5	0,6	0,2	0,0	0,0
CV, %	27,6	44,1	46,6	15,8	23,8	18,8

Seed morphology was characterized with seed length (SL), seed width (SW) and seed thickness (ST). Most of accessions formed seeds seed length > 1 mm and seed width >0.7mm. The accession with cat.No B7E0212 formed the longest seed with 1.76 mm and seed width 0.86 mm. The mean value of seed length among the studied accessions were 1.1 and seed width 0.7. Seed size was presented with 100 seed weight (g) where accession No 73293 showed the biggest seeds with 44.47 g of 100 seeds and two accessions No B7E0212 and No A9E1121 showed >39 g of 100 seeds weight. Seed morphology is characterized with less variation (Table 3) with CV -17.4% (SL) and CV-15.5% (SW) and higher coefficient of variation of seed size, compared with the previous seeds' traits with CV (100 SW) - 26%.

Table 3. Seed morphology

Cat.№	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Weight of 100 seeds (g)
A7E0269	1,06	0,58	0,5	29,8
A7E0268	1,36	0,76	0,5	30,3
A7E0270	1,18	0,78	0,56	34,1
A7E0292	1,18	0,5	0,4	28,45
A8E0271	0,96	0,76	0,5	22,7
A8E0634	1,26	0,76	0,5	35,48
A8E0543	1,24	0,74	0,5	35,4
A8E0646	1,04	0,74	0,54	37,84
A8E0641	1,08	0,68	0,5	25,84
A8E0458	1,16	0,66	0,5	28,54
A8E0439	1,14	0,7	0,488	31,974
A9E1426	1,06	0,56	0,46	33,72
A9E1123	1,3	0,7	0,5	27,7
A9E0708	1,04	0,76	0,56	31,43
A9E0639	0,84	0,54	0,5	20,06
786091B	1,1	0,7	0,5	21,4
A9E0449	1,26	0,66	0,5	30,14
A9E0405	0,9	0,74	0,58	26
A9E0311	1,17	0,6	0,5	23,73
A9E0608	1,06	0,56	0,46	37,6
B0E0056 A	1,3	0,62	0,52	35,5
A9E0587	1,3	0,68	0,58	38,87
A9E0182	1,1	0,74	0,58	34,92
78E6149A	1,34	0,58	0,5	30,27
A9E0139	1,1	0,7	0,58	28,63
A9E1121	1,06	0,76	0,66	39,34
C0E0308	1,06	0,46	0,4	16,31
A9E1008	1,26	0,66	0,46	23,59
A4E0001	1,34	0,68	0,48	29,38
A7E0668	1,34	0,68	0,48	28,9
A7E0137	1,24	0,66	0,48	33,87
811355	0,88	0,56	0,48	15,28
741356	0,86	0,48	0,48	16,8
B9E0001	0,86	0,46	0,46	20,9
B0E0054	1,16	0,66	0,56	30,55
B0E0048	0,9	0,52	0,48	19,2
741261	0,8	0,58	0,5	28,9
771174	1,2	0,6	0,5	21,47
811365	0,86	0,56	0,48	13,2
801331	1,06	0,56	0,5	23,06
771494	1,56	0,76	0,56	33,42
66720	1,24	0,74	0,5	33,6
781230	1,06	0,56	0,46	16,41
B1E0520	1,52	0,72	0,56	27,4
B1E0536	1,12	0,64	0,52	25,4
B7E0212	1,76	0,86	0,58	39
761162	0,88	0,58	0,5	23,3
78E6781A	1,24	0,64	0,5	28,2
761376	1,04	0,64	0,54	17,76
771173	1,26	0,56	0,46	15,78
781225	0,86	0,76	0,5	23,8
62258	1,04	0,64	0,48	19,81
73293	1,56	0,96	0,5	44,47
A9E0113	1,26	0,76	0,56	25,47
B0E0056B	1,16	0,56	0,46	27
Minimum	0,8	0,46	0,4	13,2

Cat.№	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Weight of 100 seeds (g)
Maximum	1,76	0,96	0,66	44,47
Mean	1,1	0,7	0,5	27,7
CV, %	17,4	15,5	9,1	26,0

Seed shape and seed color are of main importance for consumers (Sinkovič et al., 2019). Seeds showed predominantly cuboid shape and white color, however there is a diversity in seed shape with round, oval and kidney seeds with beige, brown and mottle seeds (Figures 2, 3, 4).

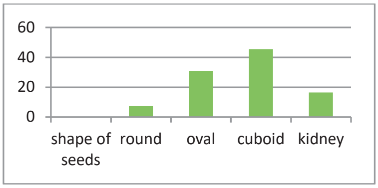

Figure 2. Seed shape

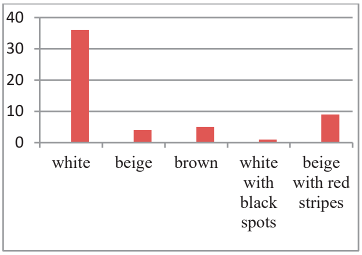

Figure 3. Seed color

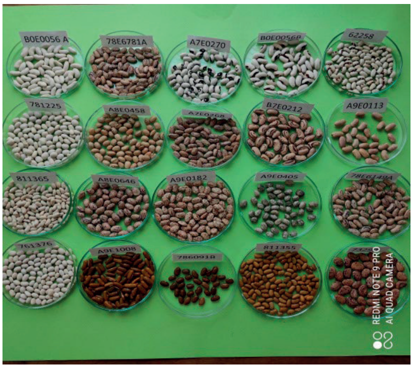

Figure 4. Diversity of *Phaseolus* seeds

Cluster analysis

Clustering *Phaseolus vulgaris* accessions based on morphological traits showed four major groups. Cluster one contains 24 accessions, cluster 2 contains 7 accessions, while cluster 3 and 4 contain 12 accessions each (Figure 5). The dendrogram showed clusters of accessions formed mainly on their origin. Genetically narrow accessions were shown by the coefficient of similarities, as follows: accession No 1 (A7E0269) with No 2 (A7E0268) and accession No 4 (A7E0292) with No 29 (A4E0001). According to the coefficients of similarities accessions with maximum genetic distance were No 53(73293) and No 39 (813965), No 22 (A9E0587) and No 39 (813965), No 16 (786091B) and No 53 (73293).

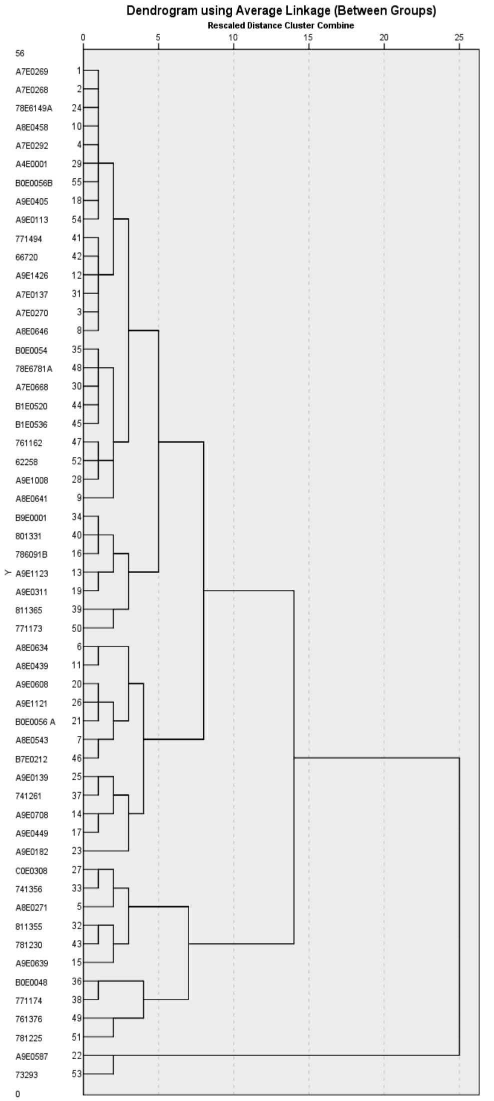

Fig. 5. Dendrogram illustrating similarities among 55 *Ph. vulgaris* accessions with local origin

During the experimental period evaluation on diseases resistance, common bacterial blight (*Xanthomonas axonopodis pv. phaseoli*) and hallo blight (*Pseudomonas syringae pv. phaseolicola*) were performed under fioeld conditions. Observations were done twice, during flowering and podding stages, using the scale from 1 to 9 (Bioversity 1982; Kiryakov & Genchev 2005).

Most of studied accessions didn't show symptoms of the two bacterial diseases (63.6%). Some of accessions showed medium resistance with 3-5 score 32.7%, while 3.6% showed sensitive reaction with evaluation of 7 scores.

The results were on the second observation, in middle of July, during experimental period. It is important to mention that from May to July there were unfavorable meteorological conditions with less quantity of rainfall than normal and it didn't allow the development of pathogens 'symptoms.

CONCLUSIONS

Quantitative traits have been used to study morphological diversity and important agronomic traits of *Ph. vulgaris* accessions with Bulgarian origin. Accessions with cat. No A7E0668 and B9E0001 were the earliest matured, while accessions cat. No 811355, cat. No 741356, cat. No 761376, cat. No 62258 needed 98 days to reach maturity.

Morphological traits, h of plant, weight of plant and weight of plant/without pods were with highest value of coefficient of variation. Accessions with cat. No A8E0641, A9E0311, A9E0182, 771174, 801331, B7E0212, 761376, 73293 were with the biggest vegetative mass of plant and showed biggest weight of plant without pods.

Diversity of seeds expressed by seed shape, seed color, and seed size was noticed with different shapes, diverse color of seeds and predominantly with medium seed size. Accession No 73293 showed the biggest seeds with 44.47 g of 100 seeds.

Evaluation on diseases resistance, common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and hallo blight (*Pseudomonas syringae pv. phaseolicola*), 32.7% of accessions showed medium resistance to the pathogens (3-5 score).

The results from this study could be used from breeders and researchers for different purposes of bean improvement. Evaluation of diseases resistance will help to select accessions with better performance under field conditions.

ACKNOWLEDGEMENTS

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SYMBIOSIS AND ECOLOGICAL ADAPTATION OF THE COMMERCIAL NITROGEN FIXING BACTERIA ON UNDERUTILISED BAMBARA GROUNDNUT CROP

Rivoningo UBISI, Gugu MASEKO, Thobile MKHWANAZI, Mmagadima SEBATI, Moses TIMANA, Nicholus MNYAMBO, Lerato KGOTSE, Elizabeth KOLA, Zakheleni DUBE

University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa

Corresponding author email: rubisi0910@gmail.com

Abstract

The efficiency of the natural symbiotic relationship between communally produced legumes and nitrogen fixing bacteria has been reported to be very poor, mainly due to poor soils in communal farming areas. With the use of synthetic fertilizers to increase soil fertility being both environment-unfriendly and unaffordable for majority of communal farmers, strategies that are cost-effective and climate smart need to be put in place to improve the productivity of communal legumes such as Bambara groundnuts. A 5 x 3 factorial arrangement experiment in a randomised complete block design with five replications was established under greenhouse conditions. Factors consisted of bacterial species: [Bradyrhizobium japonicum (Bj), Rhizobium loti (Rl), Rhizobium meliloti (Rm), Rhizobium leguminosarum biovar phaseoli (Rlt) and a negative control (untreated)], and local Bambara groundnut varieties: black, creamy white-eye, and red. Bambara groundnut seeds were inoculated with levels recommended for cowpea and soyabean. Nitrogen fixing bacteria application on Bambara groundnuts increased plant growth and nodulation variables, with the effects varying with Bambara groundnut variety and rhizobium type. Bj outperformed the other tested bacteria on nodulation variables, with the red cultivar performing better in all growth variables, making it suitable for pod and vegetative production.

Key words: *Bambara groundnut, Bradyrhizobium japonicum, Nitrogen fixing bacteria, Inoculum, Rhizobium spp., soil fertility.*

INTRODUCTION

An ever-growing population and climate change are the main factors that have negatively impacted agricultural production (Olabanji et al., 2020). The two calls for the utilization of an increased number of food crops which were previously deemed underutilized to ensure that food security is met (Cook, 2017). Bambara groundnut (*Vigna subterranea* L.) is an underutilized, indigenous, drought-tolerant legume crop mostly grown by subsistence farmers in sub-Saharan Africa for its grain. It is the third most significant food legume in Africa after cowpea (*Vigna unguiculata*) and groundnuts (*Arachis hypogaea*) both in land cultivation and consumption (Ibny et al., 2019). The edible grain of this legume contains high carbohydrates (55.6%), protein (21.2%), fats (7%), and fibre (6.3%), which makes it a comprehensive meal (Pouzaa et al., 2017). Due to Bambara groundnut's great nutritional value, the crop is labelled as ‘a native key solution to African’s food crisis’ (Temegne et al., 2018). Harvested seeds of Bambara groundnut, can be consumed fresh or dried for future consumption (Cook, 2017). The leaves contain high K and N, therefore can serve as an excellent protein rich feed for animals.

Low soil nitrogen (N) is a major constraint for sustainable crop production in communal farming systems in Africa (ref). Farmers mostly rely on synthetic fertilizers to improve soil fertility and these fertilizers are environment-unfriendly and very expensive for the smallholder farmers who are resource-poor (Gomoung et al., 2017). Due to colonial history, most communal farmers who depend on the crop are found in degraded soils very low in nutritional status and a natural population of the beneficial organisms resulting in reduced crop productivity (Hasan et al., 2018; Nyamador et al., 2016).

Grain legumes such as Bambara groundnuts can form symbiotic association with nitrogen fixing root nodule bacteria called ‘rhizobia’; in a process that can supply sufficient N for the legume and other crops growth in the same land under intercrop or crop rotation (Azman-Halimi et al., 2019; Ibrahim et al., 2018). This symbiotic relation is mainly of economic and agronomic significance due to its N contribution to the total balance of N in the terrestrial ecosystems (Stagnari et al., 2017). Most of the research on the symbiotic relationship between Bambara groundnut and nitrogen-fixing bacteria have focused on its promiscuous tendencies to pick bacteria in soils (Akpalu et al., 2013).

The compatibility of Bambara groundnut with commercially produced nitrogen-fixing bacterial could help improve the yields of the crop and quality of the soils and are less expensive, when compared to synthetic fertilisers, at the same time promote sustainable farming (Nelwamondo, 2020). Hence the study sought to determine the effectiveness and adaptability of the comercially produced nitrogen-fixing bacteria on Bambara groundnuts growth and nodulation.

MATERIALS AND METHODS

Description of the study site

The study was conducted in a greenhouse located at the University of Mpumalanga farm (25°27′06.18″S 30°58′5.21″E) Mbombela campus, South Africa. The temperature and humidity in the greenhouse were set at 25 ± 2°C and 70 ± 10%, respectively.

Experimental treatments and design

The experimental treatments were arranged in a 5 x 3 factorial arrangement in a Randomised Complete Block Design (RCBD) with five replications (Figure 1).

Figure 1: Experimental layout in greenhouse

The first factor comprised of five bacterial inocula: *Bradyrhizobium japonicum* (Bj), *Rhizobium loti* (Rl), *Rhizobium meliloti* (Rm), and *Rhizobium leguminosarum* biovar phaseoli (Rlt) inoculum at a manufacturer recommended rates for cowpeas and soyabean, and a negative control (untreated seeds), whereas the second factor was the Bambara groundnut varieties; black (C1), and creamy white-eye (C2), and red (C3).

A 25 cm diameter pots were filled with steam-pasteurised sandy loam soil. Before inoculation, seeds were mixed with 48% sugar solution in order to get a thin uniform coating of inoculum before sowing. A 15 g of seeds was used for each inoculation following manufacturer's recommendation of 6.5 x 10^7 live cells/seed of *Bradyrhizobium japonicium*, 5 x 10^7 live cells/seed of *Rhizobium loti* and 2.4 x 10^7 live cells/seed of *Rhizobium leguminosarum* biovar *phaseoli* and *Rhizobium meliloti*. Seeds were sown an hour after inoculation and were irrigated with 300 ml of tap water per plant when soil moisture levels were below 50 %. Monitoring of seed germination and scouting for pests was done daily.

Data collection

Plant growth variables

Seedling emergence and days to flowering were recorded every day from the first sign until 100%. After 110 days, the number of leaves and number of runners were counted per plant and recorded. Plant height measured from the soil level to the tip of the flag leaf using a ruler. Length of the longest runner was measured using a 30 cm ruler. Chlorophyll content was measured from two mature topmost leaves utilizing a Chlorophyll Content Meter. The plants were then cut at soil line and stem diameter measured using Vernier Callipers (C.C, Johannesburg) 5 cm from the severed end. Plant roots and pods were removed from the pots immersed in water to wash off soil particles and blotted dried using paper towel and number of fresh pods per plant were recorded, the weight per plant of fresh pod, shoot, and roots per plant were measured and recorded using electronic weighing balance. The shoots were then oven-dried for 72 hours at 55 °C and weighed to determine dry shoot mass (DSM). Plant growth vigour score as a measure of plant nitrogen access levels was assessed based on the following previously described scoring system (Corbin et al., 1977) where plants that are vigorous and green were scored at 5, those relatively small and green were given a score of 4, plants that were losing the green pigment and were small had a score of 3, plants that were slightly chlorotic had a score of 2, and finally plants that were very chlorotic were given a score of 1.

Plant Nodulation variables

Abundance and presence of nodules were assessed from the root system of each plant. The number of active nodules (strong pink internal colour), and nonactive (brown, green, or white internal colour) were taken. Nodule colour and abundance were assessed using a previously described scoring system (Somasegaran & Hoben, 1994; Corbin et al*.,* 1977), where more than five clusters of pink pigmented nodules were scored at 5, four to five clusters of mostly pink nodules were scored 4, three to four cluster of less pink nodules 3, some pink or whitish with green area 2, less than three clusters of nodules or green or white nodules 1, and roots with no nodules or green or white nodules were scored 0. Nodule position score was assessed as follows: where there were both crown and lateral nodulation a score of 3 was given, crown nodulation only 2, and where lateral nodulation only occurred a score of 1 was given. Nodulation efficiency and nitrogen fixation potential were determined by adding the scores for plant vigour, nodule colour & abundance score, and nodule position score. Nodulation efficiency and nitrogen fixation potential were then rated using total scores as follows: 11-13 score indicate excellent nodulation and nodule nitrogen fixing potential, 7-10 score indicate good still effective nodulation with nodules having limited nitrogen fixing potential, 1-6 score indicated poor nodulation with nodules with very little to no nitrogen-fixation potential.

Data analysis

Data collected were tested for normal residual distribution using the Shapiro-Wilk normality test before subjecting it to analysis of variance (ANOVA) (Gomez & Gomez, 1984). The data that were not normally distribution were transformed using $Log_{10}(x+1)$ then subjected to ANOVA through Statistix 10 software. The mean separation was achieved using Fisher's Least Significant Differences (LSD) at 5% probability level.

RESULTS AND DISCUSSIONS

Shapiro-Wilk test for normality distribution revealed that most plant growth variables and all nodulation variables were normally distributed ($P \leq 0.05$), except for plant height, length of longest runner, fresh shoot mass, dry shoot mass, and fresh root mass hence were transformed accordingly. The interaction between Bambara groundnut variety and bacteria inoculum were not significant in all tested variables.

Plant growth variables

Rhizobia inoculation is an important technology used to address soil fertility problems and application of insufficient fertilizer in legume production (Saharan & Nehra, 2011). Effective application of rhizobia strains as bio-fertilizers to improve production is a significant approach in sustainable agriculture (Saharan & Nehra, 2011). Glasshouse inoculation experiments on three Bambara groundnut varieties with four isolates and one reference strains (bacteria in untreated soil) from this study resulted in varying effects on all tested variables. The current study reports that there were no interactions between varieties and bacteria inoculum. However, bacteria treatments had highly significant effects on the number of leaves, fresh shoot mass, and dry shoot mass, contributing 43, 48, and 47% to the total treatment variable (TTV) (Table 1). Moreover, bacteria also had significant effect on stem diameter and fresh root mass, contributing 22 and 24% to TTV, respectively (Table 1). varieties had highly significant effect on the number of runners, length of longest runner, number of leaves, and pod mass, contributing 75, 81, 39, and 78% ($P \leq 0.01$) (Table 1).

The black variety was statistically different from the red and creamy white-eye varieties on number of runners, length of the longest runner, chlorophyll content, pod mass, and number of leaves expect for the number of pods which was similar to the red variety (Table 2). Additionally, the black variety had the highest pod number, pod mass, and chlorophyll content whereas the red variety had the least of the same variables (Table 2). The red variety had the highest number of runners with the black variety having the least. Bacteria in untreated soil outperformed the commercial inoculum in all plant growth variables (Table 3). The inferiority of the seed coated with commercial rhizobacteria inoculant could be attributed to the texture and big seed size of Bambara groundnut relative to cowpea and soyabean were these are recommended. Bambara groundnut seeds might not have imbibed optimum quantity of the liquid inoculum suspension that is capable of effectively promoting plant growth. Milus and Rothrock (1993) also noted that the bacteria population in the soil dependent on the initial stack of inoculums on the seeds coat. In contrary, Nelwamondo (2020) observed that inoculation of cowpea with *Bacillus subtills* strain BD233 *and Bradyrhizobium japonicum* significantly increased number of leaves and plant fresh weight, having a weight of 175.18 g, followed by *Bradyrhizobium japonicum* inoculation having a plant fresh weight of 165.38 g. Allito et al. (2015) reported that the response of legumes to inoculum differs with the degree of promiscuity of legume species. These results were in line with Yakubu et al. (2011), Manisha and Bhadoria (2008), who observed significant responses of groundnut to Rhizobia inoculation.

Nodulation variable

All measured variables were significant ($P \leq 0.05$), except for plant growth vigor and total assessment score. Bacteria treatments had highly significant ($P \leq 0.01$) effects on the total number of nodules and non-active nodules contributing 80% and 78% in TTV (Table 4). Bacteria treatments also had significant ($P \leq 0.05$) effects on nodule position, number of active nodules, and nodule color and abundance score, contributing 46, 66, 48% in TTV of the respective nodulation variables (Table 4). Inoculation of Bambara groundnut seeds resulted in a significant variation in all nodulation variables particularly in enhanced nodule formation (Figure 2A) (Table 5).

All treatments that were inoculated with *Bradyrhizobium japonicum* isolate had high number of nodules but with less pink internal nodule colours and accumulation of plant biomass (Table 5). These results indicate a higher rate of symbiotic efficiency and N-fixation. The current observation support report by Onyango and Ogolla (2019), that *Bradyrhizobium* spp. isolate may have acquired both *nif* and *nod* genes within the *Sym* plasmid which enabled it to produce the high number of nodules. Bacteria isolates in untreated soil had the highest number of active nodules when compared to the commercial inoculum (Figure 2B) (Table 5).

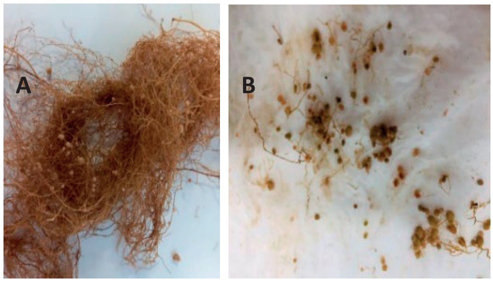

Figure 2. Nodules on roots of Bambara groundnut (A); Interior colour of the nodules (B)

McLoughlin et al. (1984) reported that inoculation efforts failed to enhance legume productivity because the indigenous strains inhabited the root nodules rather than the inoculum strains. This was related to a more competitive nature and adaptability of the indigenous population. *Rhizobium meliloti*, *Rhizobium loti*, and *Rhizobium leguminosarum* had higher number of active nodules, colour and abundance of the nodules (Table 5). On the number of active nodules *Rhizobium meliloti*, *Rhizobium loti*, and *Rhizobium leguminosarum* were statistically different from *Bradyrhizobium japonicum* and isolates from untreated soil (Table 5). These strains have the potential to be use as bio-fertilizers in Bambara groundnuts production, hence they well distributed in most soil types. Ikenganyia, Anikwe and Ngwu (2018) stated that when bacteria isolates are introduced into the rhizosphere, they have the potential to change microbial populations in the rhizosphere and influence nutrient availability, transformation, and uptake by plants. Furthermore, *Rhizobium* and *Agrobacterium* are known as the fast-growing genera which have better rates of competition for nodulation occupancy (Howieson, Nutt, and Evans, 2000). Consequently, Bambara groundnuts are mostly more compatible with the genus *Bradyrhizobium* and *Burkholderia* spp. but are outcompeted for nodulation colour and abundance as well as the total nodulation for effectiveness in the local soils by the *Rhizobium* spp. Conversely, the ability of the isolates to thrive and result in more nodule occupancy, and interior pink colour is influenced by the prevailing status of the soil (Slattery, Pearce and Slattery, 2004).

Table 1: Partitioning mean sum of squares for number of runners (NR), length of longest runner (LLR), chlorophyll content (CC), number leaves (NL), stem diameter (SD), pod number (PN), pod mass (PM), fresh shoot mass (FSM), dry shoot mass (DSM) and fresh root mass (FRM) for Bambara groundnut growth in response to cultivar and commercial nitrogen fixing bacteria.

		NR		LLR		CC		NL		SD	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.163	17	8.170	4	0.008	4	27.180	8	2.949×10^{-3}	53
varieties	2	0.732	75^{***}	149.467	81^{***}	0.109	53^{**}	127.960	39^{***}	8.762×10^{-4}	16^{ns}
Bacteria	4	0.034	3^{ns}	7.300	4^{ns}	0.054	26^{ns}	140.313	43^{***}	1.243×10^{-3}	22^{**}
Varieties*Bacteria	8	0.014	1^{ns}	6.691	4^{ns}	0.004	2^{ns}	8.043	2^{ns}	1.928×10^{-4}	3^{ns}
Error	56	0.036	4	12.776	7	0.030	15	26.544	8	3.279×10^{-4}	6
Total	74	0.980	100	184.403	100	0.206	100	330.04	100	5.589×10^{-3}	100
		PN		PM		FSM		DSM		FRM	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.200	10	0.047	-6	38.076	31	5.741	41	86.751	61
Varieties	2	1.203	61^{**}	0.064	78^{***}	10.993	9^{ns}	0.412	3^{ns}	1.145	1^{ns}
Bacteria	4	0.301	15^{ns}	0.077	9^{ns}	58.647	48^{***}	6.522	47^{***}	33.556	24^{**}
Varieties* Bacteria	8	0.075	4^{ns}	0.016	2^{ns}	6.718	5^{ns}	0.339	2^{ns}	7.699	5^{ns}
Error	56	0.178	9	0.038	5	7.802	6	0.933	7	13.629	10
Total	74	1.957	100	0.823	100	122.236	100	13.950	100	142.780	100

^{ns}Not significant at $P > 0.05$, **Significant at $P \leq 0.05$, ***Highly significant at $P \leq 0.01$. $^{\times10}$ Exponents beginning with three zeros before the comma.

Table 2: Effect of varieties on number of runners (NR), length of longest runner (LLR), chlorophyll content (CC), pod number (PN), pod mass (PM), and number of leaves (NL) for Bambara groundnut.

Varieties	NR	LLR	CC	PN	PM	NL
Cream-white eye	0.805^{a}(5.720)	9.912^{a}	1.288^{b}(19.684)	0.263^{b}(1.400)	0.031^{c}(0.078)	17.000^{a}
Red variety	0.818^{a}(5.800)	8.568^{a}	1.286^{b}(19.492)	0.571^{a}(5.000)	0.164^{b}(0.624)	16.560^{a}
Black variety	0.515^{b}(2.960)	5.168^{b}	1.402^{a}(26.284)	0.688^{a}(6.520)	0.350^{a}(1.716)	12.880^{b}
F-value	20.210	11.700	3.620	6.750	16.860	4.820
P-value	0.000	0.001	0.033	0.002	0.000	0.012
$LSD_{0.05}$	0.108	2.025	0.099	0.239	0.1108	2.919

Values that are found in brackets are means which are not transformed [$Log_{10}(x+1)$].

Table 3: Effect of *Bradyrhizobium japonicum* and *Rhizobium* spp. on the number of leaves (NL), stem diameter (SD), fresh shoot mass (FSM), dry shoot mass (DSM), and fresh root mass (FRM) for Bambara groundnut cultivars.

Bacteria	NL	SD	FSM	DSM	FRM
5	20.267^{a}	0.223^{a}	12.432^{a}	4.659^{a}	16.223^{a}
4	11.733^{b}	0.157^{c}	7.185^{c}	2.876^{c}	13.799^{ab}
3	15.000^{b}	0.191^{abc}	8.234^{bc}	3.299^{bc}	14.637^{ab}
2	15.400^{b}	0.197^{ab}	9.823^{b}	3.666^{b}	12.299^{b}
1	15.000^{b}	0.171^{bc}	9.465^{b}	3.645^{b}	13.179^{b}
F-value	5.290	3.790	7.520	6.990	2.460
P-value	0.001	0.009	0.001	0.000	0.056
$LSD_{0.05}$	3.769	0.0132	2.043	0.707	2.700

1= *Bradyrhizobium japonicum*, 2= *Rhizobium loti*, 3= *Rhizobium leguminosarum biovar phaseoli*, 4= *Rhizobium meliloti*, and Control.

Nodulation variable

All measured variables were significant ($P \leq 0.05$), except for plant growth vigor and total assessment score. Bacteria treatments had highly significant ($P \leq 0.01$) effects on the total number of nodules and non-active nodules contributing 80% and 78% in TTV (Table 4). Bacteria treatments also had significant ($P \leq 0.05$) effects on nodule position, number of active nodules, and nodule color and abundance score, contributing 46, 66, 48% in TTV of the respective nodulation variables (Table 4). Inoculation of Bambara groundnut seeds resulted in a significant variation in all nodulation variables particularly in enhanced nodule formation (Figure 2A) (Table 5).

All treatments that were inoculated with *Bradyrhizobium japonicum* isolate had high number of nodules but with less pink internal nodule colours and accumulation of plant biomass (Table 5). These results indicate a higher rate of symbiotic efficiency and N-fixation. The current observation support report by Onyango and Ogolla (2019), that *Bradyrhizobium* spp. isolate may have acquired both *nif* and *nod* genes within the *Sym* plasmid which enabled it to produce the high number of nodules. Bacteria isolates in untreated soil had the highest number of active nodules when compared to the commercial inoculum (Figure 2B) (Table 5).

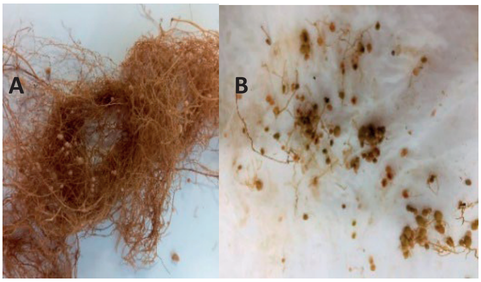

Figure 2. Nodules on roots of Bambara groundnut (A); Interior colour of the nodules (B)

McLoughlin et al. (1984) reported that inoculation efforts failed to enhance legume productivity because the indigenous strains inhabited the root nodules rather than the inoculum strains.

This was related to a more competitive nature and adaptability of the indigenous population. *Rhizobium meliloti*, *Rhizobium loti*, and *Rhizobium leguminosarum* had higher number of active nodules, colour and abundance of the nodules (Table 5).

On the number of active nodules *Rhizobium meliloti*, *Rhizobium loti*, and *Rhizobium leguminosarum* were statistically different from *Bradyrhizobium japonicum* and isolates from untreated soil (Table 5).

These strains have the potential to be use as bio-fertilizers in Bambara groundnuts production, hence they well distributed in most soil types. Ikenganyia, Anikwe and Ngwu (2018) stated that when bacteria isolates are introduced into the rhizosphere, they have the potential to change microbial populations in the rhizosphere and influence nutrient availability, transformation, and uptake by plants.

Furthermore, *Rhizobium* and *Agrobacterium* are known as the fast-growing genera which have better rates of competition for nodulation occupancy (Howieson, Nutt, and Evans, 2000). Consequently, Bambara groundnuts are mostly more compatible with the genus *Bradyrhizobium* and *Burkholderia* spp. but are outcompeted for nodulation colour and abundance as well as the total nodulation for effectiveness in the local soils by the *Rhizobium* spp.

Conversely, the ability of the isolates to thrive and result in more nodule occupancy, and interior pink colour is influenced by the prevailing status of the soil (Slattery, Pearce and Slattery, 2004).

Table 4: Partitioning mean sum of squares for nodule position (NP), nodule number (NN), active nodules (AN), non-active nodules (NNA), and nodule color and abundance (NCA) for Bambara groundnut nodulation in response to cultivars and commercial nitrogen-fixing bacteria

Source	NP MSS	NP TTV (%)	NN MSS	NN TTV (%)	NA MSS	NA TVV (%)	NNA MSS	NNA TTV (%)	NCA MSS	NCA TTV (%)
Replication	0.041	26	0.069	7	0.049	4	0.193	10	0.056	18
Varieties	0.006	4^{ns}	0.002	0^{ns}	0.085	7^{ns}	0.028	1^{ns}	0.024	8^{ns}
Bacteria	0.074	46^{**}	0.747	80^{***}	0.779	66^{**}	1.576	78^{***}	0.149	48^{**}
Varieties*Bacteria	0.024	15^{ns}	0.054	6^{ns}	0.161	13^{ns}	0.103	5^{ns}	0.051	17^{ns}
Error	0.015	9	0.060	6	0.116	10	0.118	6	0.032	10
Total	0.160	100	0.930	100	1.190	100	2.020	100	0.310	100

^{ns}Not significant at $P > 0.05$, **Significant at $P \leq 0.05$, ***Highly significant at $P \leq 0.05$

Table 5: Effect of commercial nitrogen-fixing bacteria on nodule position (NP), number of nodules (NN), number of active nodules (AN), number of non-active nodules (NNA), nodule colour and abundance (NCA) for Bambara groundnut cultivars

Bacteria	NP	NN	AN	NNA	NCA
5	0.442^{b}(1.933)	1.693^{b}(50.870)	1.400^{a}(25.867)	1.369^{b}(25.000)	0.778^{a}(5.000)
4	0.453^{b}(2.000)	1.514^{c}(35.930)	1.107^{b}(13.400)	1.258^{b}(22.530)	0.758^{a}(4.800)
3	0.457^{b}(2.000)	1.596^{bc}(43.870)	1.042^{b}(13.400)	1.379^{b}(30.470)	0.686^{a}(4.133)
2	0.433^{b}(1.867)	1.519^{bc}(35.270)	1.044^{b}(12.600)	1.235^{b}(22.600)	0.721^{a}(4.467)
1	0.602^{a}(3.000)	2.052^{a}(135.600)	0.759^{c}(9.667)	2.021^{a}(125.930)	0.527^{b}(3.267)
F-value	4.770	12.420	6.700	13.330	4.620
P-value	0.002	0.000	0.000	0.000	0.003
$LSD_{0.05}$	0.091	0.179	0.249	0.252	0.132

1= *Bradyrhizobium japonicum*, 2= *Rhizobium loti*, 3= *Rhizobium leguminosarum biovar phaseoli*, 4=*Rhizobium meliloti,* 5= Control.
Values that are found in brackets are means which are not transformed [$Log_{10}(x+1)$].

CONCLUSIONS

There is variation in the response of the different varieties of Bambara groundnuts to the different treatments with species of bacteria. The black variety could be recommended for yield production as it gave the highest pod mass and numbers, whereas the red variety can be used for vegetative production as it had the highest vegetative growth. Natural occurring bacteria outperformed the commercial species in Bambara groundnut nodulation giving the highest number of active nodules, hence identification of this bacteria is recommended for future study. *Bradyrhizobium japonicum* had the high number of nodules yet the lowest number of active nodules.

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AN OVERVIEW OF RECENTLY DISCOVERED INTRA AND INTER-GENERIC ORCHID HYBRIDS AS NEW ADDITIONS TO ROMANIAN FLORA

Nora ANGHELESCU[1], Lori BALOGH[2], Mihaela BALOGH[2], Nicoleta KIGYOSSY[2], Remus DULUGEAC[3], Hajnalka KERTÉSZ[4], Ramy MAALOUF[5], Mihaela GEORGESCU[1], Sorina PETRA[1], Florin TOMA[1], Adrian PETICILĂ[1]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464 Bucharest, Romania
[2]Association 'Comori de pe Valea Prahovei', 2 Avram Iancu St., 106100, Sinaia, Romania
[3]Police Department, Criminal Investigation Bureau, 110224, Piteşti, Romania
[4]Bethlen Gábor Middle School & Román Viktor Middle School, Odorheiu Secuiesc, Harghita County, Romania
[5]Independant researcher, Jardim Vila Mariana, São Paulo - SP, 04115-000, Brazil

Corresponding author email: noradeangelli15@gmail.com

Abstract

We describe, for the first time, fourteen of the most spectacular intra- and inter-generic orchid hybrids, new to Romanian flora. As crosses between species of the same or different genera, their occurrence is exceptionally rare in the wild, both in Romania and throughout temperate Europe. Discovering and having the chance to study them in detail in the wild, represents a remarkable privilege. During our three-year study (2020-2023), we encountered several hybrids belonging to the following genera, Anacamptis (A.× menosii; Anacamptis × olida nothosubsp. paparistoi), Ophrys (O. × minuticauda), Orchis (O. × loreziana nothosubsp. kisslingii), Neotinea (Neotinea × dietrichiana), Dactylorhiza (D. × ruppertii) and nothogenera × Dactylodenia (× D. lebrunii; × D. heinzeliana; × D. illyrica nothosubsp. siculorum; × D. sinaiensis) and × Pseudadenia (× P. schweinfurthii; × P. vitosana). Out of these, × Dactylodenia illyrica nothosubsp. siculorum (Dactylorhiza cordigera subsp. siculorum × Gymnadenia frivaldii) and × Dactylodenia sinaiensis (Dactylorhiza saccifera × Gymnadenia conopsea), are regarded as new-to-science taxons, hereby described for the first time. To give a complete overview, we included the inter-generic hybrid belonging to × Pseudorhiza nothogenus (× P. nieschalkii nothosubsp. siculorum). In addition, information regarding their distribution, habitat, ecology, phenology and IUCN conservation assessments are provided.

Key words*: orchids, intergeneric, hybrids, × Dactylodenia, × Pseudadenia, conservation, intrageneric.*

INTRODUCTION

In this article, we document the first reported natural occurrence of **six** ***intra-generic hybrids*** belonging to genera, ***Anacamptis*** - ***Anacamptis*** **×** ***menosii*** (Chr. Bernard & G. Fabre) H. Kretzschmar, Eccarius & H. Dietr., 2007 (*A. coriophora* × *A. papilionacea*), ***Anacamptis*** **×** ***olida*** **nothosubsp.** ***paparistoi*** (Gölz & H.R. Reinhard) H. Kretzschmar, Eccarius & H. Dietr., 2007 (*A. coriophora* × *A. morio* subsp. *caucasica*), ***Ophrys*** - ***Ophrys*** **×** ***minuticauda*** Duffort, 1902 (*O. apifera* × *O. scolopax* subsp. *cornuta*), ***Orchis*** - ***Orchis*** **×** ***loreziana*** **nothosubsp.** ***kisslingii*** (Beck) Potucek, 1976 (*O. mascula* subsp. *speciosa* × *O. pallens*), ***Neotinea*** - ***Neotinea*** **×** ***dietrichiana*** (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr., 2007 (*N. tridentata* × *N. ustulata*) and ***Dactylorhiza*** - ***Dactylorhiza*** **×** ***ruppertii*** (M. Schulze) Borsos & Soó, 1960 (*D. majalis* × *D. sambucina*). Additionally, we included **eight**, exquisitely rare ***inter-generic hybrids***, members of the nothogenera × ***Dactylodenia*** (*Dactylorhiza* × *Gymnadenia* genera) and × ***Pseudadenia*** (*Pseudorchis* × *Gymnadenia* genera). The chances of these specific hybridisation events occurring simultaneously are exceedingly rare: × ***Dactylodenia lebrunii*** (E.G. Camus) Peitz, 1972 (*Dactylorhiza majalis* × *Gymnadenia conopsea*); × ***Dactylodenia heinzeliana*** (Reichardt) Garay & H.R. Sweet, 1969

(*Dactylorhiza fuchsii* × *Gymnadenia conopsea*); × ***Dactylodenia illyrica* nothosubsp. *siculorum*** L. Balogh & Mih. Balogh, N. Anghelescu, N. Kigyossy, 2023 nothosubsp. *nov*. (*Dactylorhiza cordigera* subsp. *siculorum* × *Gymnadenia frivaldii*); × ***Dactylodenia sinaiensis*** N. Kigyossy, N. Anghelescu, L. Balogh & Mih. Balogh, 2023 nothosp. *nov.* (*Dactylorhiza saccifera* × *Gymnadenia conopsea*), × ***Dactylodenia* cf. *vitosensis*** Jagiello, 1988 (*Dactylorhiza cordigera* × *Gymnadenia conopsea*) and × ***Pseudadenia schweinfurthii*** (Hegelm. ex A. Kern.) P.F. Hunt, 1971 (*Gymnadenia conopsea* × *Pseudorchis albida* subsp. *tricuspis*); × ***Pseudadenia vitosana*** (H. Baumann) O. Gerbaud & W. Schmid, 1999 (*Gymnadenia frivaldii* × *Pseudorchis albida* subsp. *tricuspis*). Out of these, × *Dactylodenia illyrica* nothosubsp. *siculorum* (*Dactylorhiza cordigera* subsp. *siculorum* × *Gymnadenia frivaldii*) and × *Dactylodenia sinaiensis* (*Dactylorhiza saccifera* × *Gymnadenia conopsea*), are regarded as new-to-science taxons, hereby described for the first time. Furthermore, another unique notho-population of nine individuals of × *Pseudadenia vitosana* (*Gymnadenia frivaldii* × *Pseudorchis albida* subsp. *tricuspis*) is described for the first time, demonstrating the successful propagation and proliferation of inter-generic combinations. Additionally, an incipient nothopopulation of two × *Dactylodenia illyrica* nothosubsp. *siculorum* hybrids was recorded between 2022-2023. These two nothopopulations may be regarded as unique in Europe and represent a novel addition to the orchid flora of Romania. Regrettably, during the summer of 2023, between the 15th and 30th of June, ***two out of the total of nine × Pseudadenia vitosana inter-generic hybrids were discovered missing at the specified locations,*** within the highly protected Harghita Mădăraş area. It is crucial to emphasize that collection of rare plant taxa, such as our rare orchid hybrids from the highly protected Harghita Mădăraş ROSCI00090 area, is illegal, even when granted a study or work permit. These taxa are strictly protected not only by international law but also by regional (Romanian) regulations. Moreover, for laboratory research or deposition of herbarium vouchers, only 1-3 individual flowers and, exceptionally, one leaf may be collected from a single individual, the holotype. In recent years, photographs of the holotype(s) are also accepted as herbarium vouchers, to ensure full protection and preservation of these extremely rare and vulnerable plants.

To give a complete overview of all natural orchid hybrids reported in Romania, we included one newly described inter-generic orchid hybrid belonging to nothogenus × ***Pseudorhiza*** (*Dactylorhiza* × *Pseudorchis* genera), recently named × ***P. nieschalkii* nothosubsp. *siculorum*** nothosubsp. *siculorum* H.Kertész & N.Anghelescu, 2020 (*Dactylorhiza fuchsii* subsp. *sooana* × *Pseudorchis albida* subsp. *tricuspis*) (Anghelescu *et al*., 2021a).

The hybrids were found/studied in two major protected natural areas of Romania, **Bucegi Natural Park** ROSCI0013, Prahova County (Hedrén et al., 2022; Anghelescu et al., 2023b) and **Harghita Mădăraş**, ROSCI00090, Harghita County, as well as in various other regions, known for their rich orchid diversity, such as **Hunedoara**, **Argeş** and **Mehedinți Counties**. Over the three-year study, from **2021 to 2023**, the authors conducted extensive fieldwork in the above regions to document and map the orchid flora present within the newly discovered, remote hybridogenic zones (Anghelescu et al., 2020; 2023a; 2023b). Our observations show that the main floral features of the nothotaxa, are generally, intermediate between the parental species.

The aims of the present study are: (1) to describe in detail the main morphological characteristics of the two nothospecies (especially those of the flowers), by comparing them to the respective parental species; (2) to give information on various aspects of their biology, relevant to understanding their ecological requirements, such as distribution, habitat, communities, phenology, reproductive requirements and conservation; (3) to present information regarding the IUCN (2021) conservation status of all the taxa considered, accompanied by photographs of the holotypes.

Taking into consideration the importance and rarity of these newly discovered nothotaxa, we strongly propose the two nothopopulations as new additions to the Romanian flora.

MATERIALS AND METHODS

Sites Studied

The studies were conducted in various natural parks and protected areas:

(1) Bucegi Natural Park, Southern Carpathians, Central Romania, is a protected area included within Natura 2000 ROSCI0013, IUCN category V. It covers Prahova, Dâmbovița and Brașov Counties, with an area of ca. 32,663 ha/326.63 km^2 and the highest elevation at Omu Peak of 2,505-2,514 m. a.s.l (Ielencz, 2005).

(2) Natura 2000 protected area ROSCI00090 Harghita-Mădăraş, located in the central-eastern part of Harghita County (EU Environment, 2014) and covers an area of 13,373 hectares. It is located at an altitude between 1,500-1,800 meters a.s.l (above sea level) (Mikfalvi & Vifkori, 1979; Marcu, 1986; Cavruc, 2000). The site is a natural area covered by deciduous, coniferous and mixed forests, natural meadows, heathlands, bogs (peat bogs) and steppes together with a rich hydrographic network that consists of several lakes and watercourses (Ciocârlan 2000; 2009).

(3) Hunedoara County - the southern range of Apuseni Mountains, with the highest peak being Poienița Peak, with an elevation of 1,437 meters a.s.l. The field study included full sun to partial shade, subalpine meadows, short grasslands, forest fringes and open woodland, on calcareous substrate, up to 1,000 meters a.s.l.

(**4) Argeş County** - studies included similar habitats, dry to moist grassland (swampy areas), forest margins, meadows and pastures on calcareous substrates, up to 700-900 meters a.s.l.

(5) Mehedinți County - the species studied prefer full sun, grassy, alkaline marsh, prone to flooding (wet meadows), forest clearings, on calcareous substrates, up to 100 meters a.s.l.

Morphometric methods/comparisons

Special attention was given to the characters that proved to be taxonomically informative and those that involve the differentiating details in the morphology of the leaves, labellum and tepals. Measurements of the vegetative and floral parts were made from living plants and fresh flowers. (Anghelescu et al., 2021b).

Digital Photographic Equipment

Digital images of individual plants and floral parts were taken using Canon 5D Mark III, Nikon D3 and Nikon D850 camera bodies equipped with Nikon Micro NIKKOR 60 mm and NIKKOR 24.0-70.0 mm lenses, Venus Optics Laowa 100 mm 2X Ultra Macro. Additional equipment included a Manfrotto Tripod and Litra Torches 2.0s. Images were analysed using Adobe Photoshop® CC 2023, Zerene Stacker Software, Vers.2021-11-16 (Anghelescu et al., 2021c).

RESULTS AND DISCUSSIONS

Overcoming the premating (prezygotic) and postmating (postzygotic) isolation barriers, species boundaries might easily be disrupted, allowing cross-pollination (Marques et al., 2014). In the cases where these isolation mechanisms are not fully effective, cross-pollination leads to hybridisation (Goulet et al., 2017). Consequently, in natural conditions, hybridization represents an evolutionary engine, which breaks down the reproductive barriers that separate different species. By definition, hybridization refers to the mating or cross-breeding of two parental lineages, P1 and P2, who possess different/distinct genetic backgrounds. When P1 and P2 reproduce (P1 × P2), their genes combine in the first offspring generation (primary hybrids), known as F1 (Scopece et al., 2007). In the resulting hybrids, each genetically inherited trait/character can be exhibited as dominant (prevailing), recessive (masked) or intermediate (Ramsey et al., 2003). Consequently, this phenomenon can have profound effects on the genetic variation within populations and the evolution of species, leading to the creation of hybrids with unique combinations of traits (Marques et al., 2014). In certain instances, these hybridization events may even lead to the emergence of entirely new species (Mayr, 1942).

Description of the fourteen nothotaxa studied

The totality of all hybrid individuals resulting from the cross-breeding of the same parental taxa (natural species, not hybrids themselves) is termed a hybrid lineage or a ***nothotaxon*** (Lowry, 2008; Gill, 1989; Scopece et al., 2008).

Following is the full description of the ***fourteen intra and inter-generic orchid hybrids*** included in this study, named according to POWO (2022), Eccarius (2016; 2022). They are all terrestrial, perennial, rhizomatous autotrophic, sympodial herbaceous geophytes (Figures 1-14).

Figure 1. *Anacamptis* × *olida* nothosubsp. *paparistoi* (Gölz & H.R. Reinhard) H. Kretzschmar, Eccarius & H. Dietr., 2007

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Anacamptis coriophora*; **B., E., H.** - *Anacamptis* × *olida* nothosubsp. *paparistoi*; **C., F., I.** - *Anacamptis morio* subsp. *caucasica*. Photos © Nora E. Anghelescu, 5 May 2018, Mehedinți County

Hybrid formula: *A. coriophora* × *A. morio* subsp. *caucasica*. **Discovered:** 5 May 2018, Nora E. Anghelescu. **Locus classicus:** Mehedinți County. **Flowering time:** April-May. **Native to:** Romania, Albania. **Description:** Stem (epigeal), 21 cm, erect, purple-tinged in the upper part (inflorescence). One basal sheath, 1-3 cauline leaves, unspotted, sessile, vivid-green, acuminate, sheathing the stem. Inflorescence lax to dense, elongated raceme, floriferous. Flowers intermediate between parental species: labellum shape, median hyperpigmented markings and the strong purple shades of its margins resemble *A. coriophora*. The thicker, robust and cylindrical spurs partially resemble *A. coriophora*, but also *A. morio* subsp. *caucasica* in the upward curving of the acuminated tip. The hood is tighter, similar to that of *A. coriophora*. **Population counts:** This particular individual was discovered in 2017, in Mehedinți County, and it is the only hybrid of this type known to occur in Romania (De Angelli & Anghelescu, 2020). **Proposed conservation status:** Endangered (EN).

Figure 2. *Neotinea* × *dietrichiana* (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr., 2007

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Neotinea tridentata*; **B., E., H.** - *Neotinea* × *dietrichiana*; **C., F., I.** - *Neotinea ustulata*. Photos B., E., H. © Lori Balogh; Photos A., C., D., F., G., I. © Nora E. Anghelescu, 5 June 2023, Prahova County

Hybrid formula: *N. tridentata* × *N. ustulata*. **Discovered:** 2 June 2023, Lori & Mihaela Balogh. **Locus classicus:** Prahova County. **Flowering time:** May-June. **Native to:** Europe to Caucasus, Romania. **Description:** Stem (epigeal), 20-40 cm, erect, vivid green. 2-4 basal leaves, forming a rosette, 2-3 cauline leaves, unspotted, sessile, vivid-green, acuminate, sheathing the stem purple-tinged at the tip (resembling *N. tridentata*). Inflorescence lax to dense, 4-8(10) cm elongated raceme, very floriferous, purple-tinged (resembling a hyperchromatic *N. tridentata*), with a burnt tip (resembling *N. ustulata*). This individual presents a significantly elongated raceme, resembling *N. ustulata* parent (*N. tridentata* has a much shorter, roundish raceme). Labellum shape, as well as the labellar purple markings, which are larger, roundish and purple, resemble *N. ustulata*. Middle labellar lobe is divided into two smaller lobules resembling both *N. ustulata* and *N. tridentata* (Kretzschmar et al., 2007). The helmets are tighter, purple-coloured, resembling *N. tridentata* (in *N. ustulata*, they are dark-red to almost black-coloured). **Population counts:** single individual, discovered in June 2023, in Prahova County. **Proposed conservation status:** Endangered (EN).

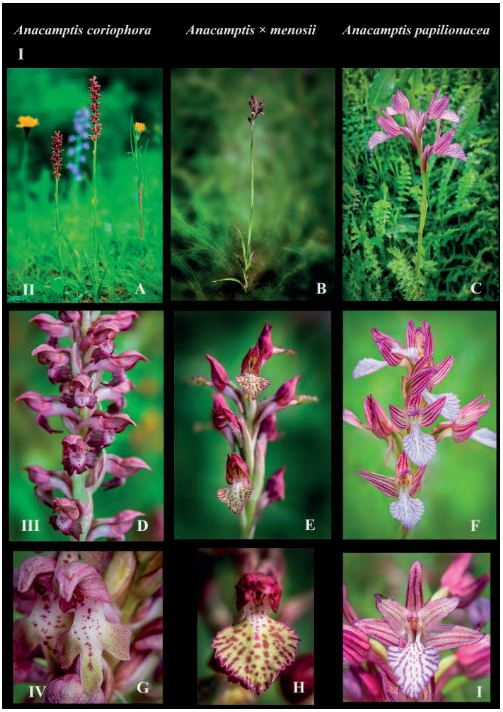


Figure 3. *Anacamptis* × *menosii* (Chr. Bernard & G. Fabre) H. Kretzschmar, Eccarius & H. Dietr., 2007
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Anacamptis coriophora*; **B., E., H.** - *Anacamptis* × *menosii*; **C., F., I.** - *Anacamptis papilionacea*. Photos © Nora E. Anghelescu, 31 May 2023, Mehedinți County

Hybrid formula: *A. coriophora* × *A. papilionacea*. **Discovered:** 31 May 2023, Nora E. Anghelescu. **Locus classicus:** Mehedinți County. **Flowering time:** May-June. **Native to:** France, Portugal, Sardegna, Romania. **Description:** Stem (epigeal), 10-14 cm, erect, vivid green, no purple pigmentation. 1-2 basal leaves, sheathing the stem, 3-5 cauline leaves, unspotted, sessile, vivid-green, acuminate, sheathing the stem (resembling *A. coriophora*; in *A. papilionacea*, the leaves are veined, purple-tinged at the tips). Inflorescence lax raceme (resembling *A. papilionacea*), less floriferous, 8-10 medium-sized flowers, resembling *A. coriophora*. Helmet deep-purple pigmented, tight, with elongated tip, resembling *A. coriophora*. Labellum entire, heart-shaped (in upper flowers, resembling *A. papilionacea*) to mildly three-lobed (in basal flowers, resembling *A. coriophora*), smaller in size than *A. papilionacea*. Labellar stripes and dots are deep-purple to red (heterosis effect) resembling *A. papilionacea*. The spur is conical, thick resembling *A. coriophora*. **Population counts:** single individual, discovered in May 2023. **Herbarium voucher specimen:** deposited at the Herbarium of the Botanical Garden Bucharest: ***BUC 410363.*** **Proposed conservation status:** Endangered (EN).

Figure 4 *Ophrys* × *minuticauda* Duffort, 1902
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail - lateral view; **A., D., G.** - *Ophrys apifera*; **B., E., H.** - *Ophrys* × *minuticauda*; **C., F., I.** - *Ophrys scolopax* subsp. *cornuta*. Photos © Nora E. Anghelescu, 16 June 2023, Bucegi Natural Park ROSCI0013, Prahova County

Hybrid formula: *O. apifera* × *O. scolopax* susp. *cornuta*
Discovered: 5 June 2021, Ramy Maalouf. **Locus classicus:** Bucegi Natural Park ROSCI0013. **Flowering time:** May-June. **Native to:** France, Greece, Crimea, North Caucasus, Sardegna, Spain, Transcaucasus, Turkey, Romania. **Description:** Stem (epigeal), 25-30 cm, erect, vivid green. 1 basal leaf, sheathing the stem, 3-5 cauline leaves, unspotted, sessile, green, acuminate (resembling *O. apifera*). Inflorescence lax raceme, less floriferous, 4-5 medium-sized flowers, resembling *O. apifera*, smaller in size than *O. scolopax* ssp. *cornuta*. Sepals light-pink, centrally green veined, resembling *O. apifera*. Lateral petals, greenish, villous (resembling *O. apifera*, triangular and equal to those of *O. scolopax* subsp. *cornuta*. Labellum three-lobed, median lobe slightly elongated, speculum bluish, resembling *O. scolopax* subsp. *cornuta*. Lateral lobes pointed,

conical-elongated, shorter, resembling *O. scolopax* subsp. *cornuta*. Basal field orange-brown, wider, resembling *O. apifera*. Appendix oblique to the labellum, intermediate between parents. Pollinia with shorter caudicle, overhanging resembling *O. apifera*. **Population counts:** single individual. **Herbarium voucher specimen:** deposited Herbarium of the Botanical Garden Bucharest: ***BUC 410370.*** **Proposed conservation status:** Endangered (EN).

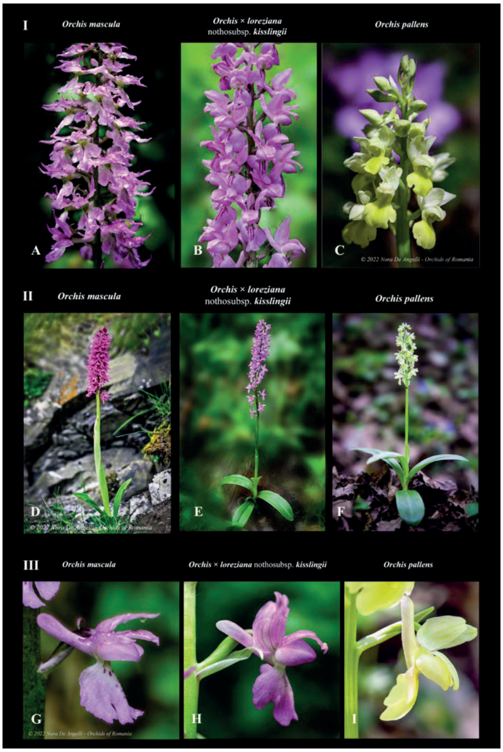


Figure 5. *Orchis* × ***loreziana* nothosubsp. *kisslingii* (Beck) Potucek, 1976**
I. Inflorescences details; **II.** Full plants in their natural habitats; **III.** Individual flower detail - lateral view; **A., D., G.** - *Orchis mascula* subsp. *speciosa*; **B., E., H.** - *Orchis* × *loreziana* nothosubsp. *kisslingii*; **C., F., I.** - *Orchis pallens*. Photos © Nora E. Anghelescu, 5 May 2023, Hunedoara County

Hybrid formula: *O. mascula* susp. *speciosa* × *O. pallens*.
Discovered: 5 May 2021, Nicoleta Kigyossy, Camelia & Cornel Alexandru. **Locus classicus:** Hunedoara County **Flowering time:** May-June. **Native to:** Romania, Austria, Czechoslovakia, Crimea. **Description:** Stem (epigeal), 20-55 cm, erect, purple-tinged in the upper part (inflorescence). 3-4 basal leaves, forming a rosette, 1-3 cauline leaves, unspotted (3 hybrids - resembling *O. pallens*) and basally purple spotted (2 hybrids - resembling *O. mascula* subsp. *speciosa*), sessile, vivid-green, acuminate, sheathing the stem. Inflorescence lax to dense, 10-20 cm raceme, floriferous (resembling *O. mascula* subsp. *speciosa*). Labellum shape, purple colour and hyperpigmented parallel stripes resemble *O. mascula* subsp. *speciosa* parent. Median and lateral lobes scalloped, intermediate Spur near-perpendicular to labellum, yellowish-purple, resembling *O. pallens*. The green parallel elongated stripes on lateral and median sepals resemble *O. pallens*. **Population counts:** ***Nothopopulation of five hybrids***. **Herbarium voucher specimen:** deposited at the Herbarium of the Botanical Garden Bucharest: ***BUC 410372.*** **Proposed conservation status:** Endangered (EN).

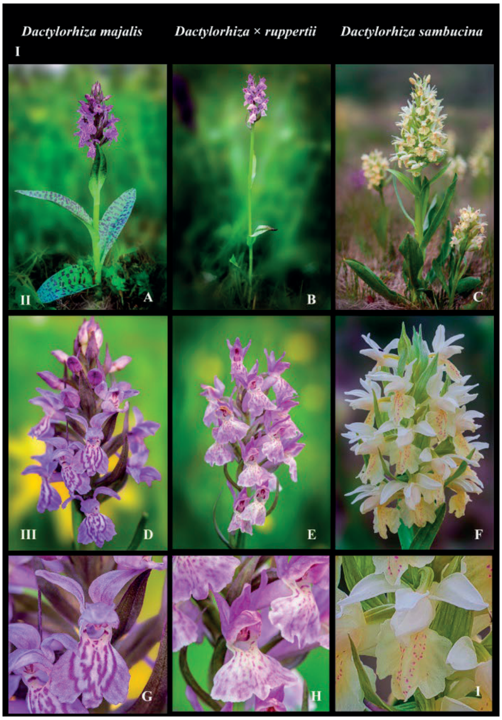


Figure 6. *Dactylorhiza* × ***ruppertii* (M. Schulze) Borsos & Soó, 1960**
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza majalis*; **B., E., H.** - *Dactylorhiza* × *ruppertii*; **C., F., I.** - *Dactylorhiza sambucina*. Photos © Nora E. Anghelescu, 10 June 2023, Harghita County

Hybrid formula: *D. majalis* × *D. sambucina*. **Discovered:** 10 June 2021, Hajnalka Kertész. **Locus classicus:** Harghita County. **Flowering time:** May-June. **Native to:** Austria, Czechoslovakia, France, Germany, Switzerland, Romania. **Description:** Stem (epigeal), 12-25 cm, erect, vivid green, mildly pigmented at the tip (resembling *D. majalis*). 1 basal leaf, sheathing the stem, 2-3 cauline leaves, sessile, vivid-green, acuminate, narrow-elongated, sheathing the stem (resembling *D. sambucina*), with minute purple maculae at the tips, resembling *D. majalis*. Inflorescence dense raceme, less floriferous, resembling *D. majalis*. Flowers large resembling *D. sambucina*. Labellum widely spread (resembling *D. sambucina*) and purple-pink coloured with mildly circular purple loops resemble *D. majalis*. Labellar central purple-pink dots and yellowish base resemble *D. sambucina*. Spur thicker, sacciform resembling more *D. sambucina*. Basal flower bracts longer than the flowers, purple pigmented, resembling *D. majalis*. **Population counts:** ***Nothopopulation of five hybrids***, including f**our dark-red hybrids between *D. majalis* × *D. sambucina* f. *rubra*. Herbarium voucher specimen:** deposited Herbarium of Botanical Garden Bucharest: ***BUC 410367.*** **Proposed conservation status:** Endangered (EN).

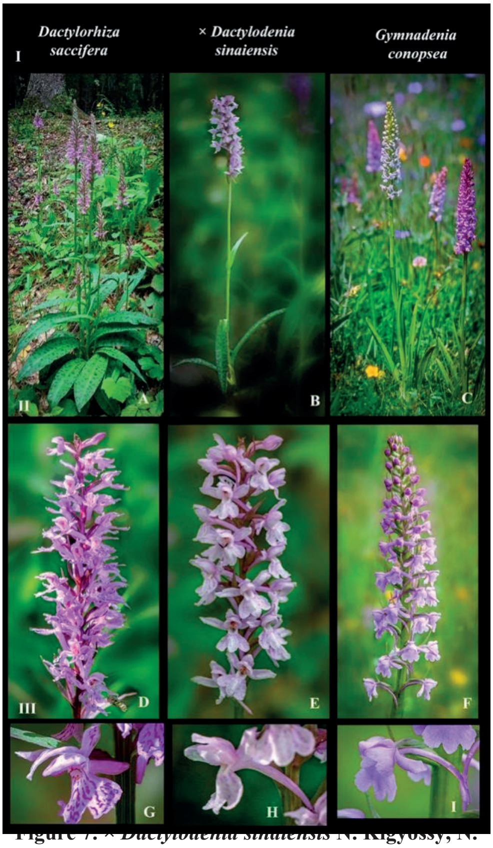
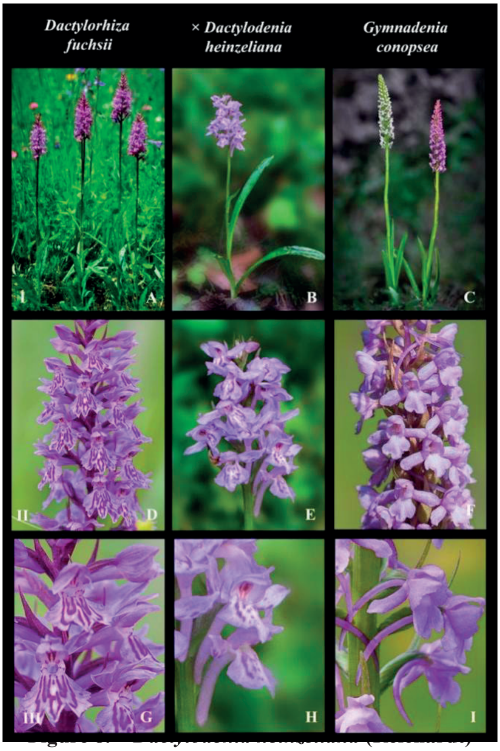


Figure 7. × *Dactylodenia sinaiensis* N. Kigyossy, N. Anghelescu, L. Balogh & Mih. Balogh, 2023 nothosp. *nov.*

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza saccifera*; **B., E., H.** - × *Dactylodenia sinaiensis*; **C., F., I.** - *Gymnadenia conopsea*. Photos © Nora E. Anghelescu, 10 July 2023, Bucegi Natural Park ROSCI0013, Prahova County

Hybrid formula: *D. saccifera* × *G. conopsea*. **Discovered:** 2 July 2023, Nicoleta Kigyossy. **Locus classicus:** Bucegi Natural Park. **Flowering time:** June-July. **Native to: Romania, *endemic*, *nothosp. nov.* Description:** Stem (epigeal), 22-24 cm, erect, vivid green, pigmented at the tip, resembling *D. saccifera*. 1 basal, 3 cauline leaves, purple-pigmented, arched, oval-acuminate resembling *D. saccifera*. Upper cauline leaf, vivid green, narrow-lanceolate. Inflorescence dense raceme, 6-7 cm, floriferous, cylindrical, resembling *D. saccifera*. Flowers pinkish, medium-sized, 7-9 mm diameter. Lateral sepals wide-spread (resembling *G. conopsea*), dorsal sepal and lateral petals forming a loose hood, resembling *D. saccifera*. Labellum three-lobed, equal in size, resembling *G. conopsea*, marked with purple stripes resembling *D. saccifera*. Spur elongated, twice as ovary, pointed similar to *G. conopsea*. **Population counts:** single individual. **Herbarium voucher specimen:** deposited Herbarium of the Botanical Garden Bucharest: ***BUC 410366.*** **Proposed conservation status:** Endangered (EN).



Garay & H.R. Sweet, 1969

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza fuchsii*; **B., E., H.** - × *Dactylodenia heinzeliana*; **C., F., I.** - *Gymnadenia conopsea*. Photos B., E., H. © Remus Dulugeac, 01 July 2023, Argeș County; Photos A., C., D., F., G., I. © Nora E. Anghelescu, 10 July 2023, Bucegi Natural Park.

Hybrid formula: *D. fuchsii* × *G. conopsea*. **Discovered:** 1 July 2023, Remus Dulugeac. **Locus classicus:** Argeș County. **Flowering time:** June-July. **Native to:** Austria, Belgium, Czechoslovakia, France, Germany, Great Britain, Ireland, Netherlands, Poland, Switzerland, Yugoslavia, Romania. **Description:** Stem (epigeal), 10(12) cm, erect, vivid green, purple-tinged at the tip, resembling *D. fuchsii*. 2 basal leaves, 2-3 cauline leaves, purple-pigmented, arched, oval-acuminate resembling *D. fuchsii*. Upper cauline leaf, vivid green, narrow-lanceolate, non-pigmented. Inflorescence dense raceme, less floriferous, flowers pinkish, medium-sized, 7-9 mm diameter, resembling *D. fuchsii*. Lateral sepals wide-spread (resembling *G. conopsea*), dorsal sepal and lateral petals forming a loose hood, resembling *D. fuchsii*. Labellum three-lobed, lateral lobes scalloped (resembling *G. conopsea*), median lobe pointed, marked with purple loops and stripes resembling *D. fuchsii*. Spur longer than the ovary, pointed downwards, resembling *G. conopsea*. **Population counts:** single individual. **Proposed conservation status:** Endangered (EN).

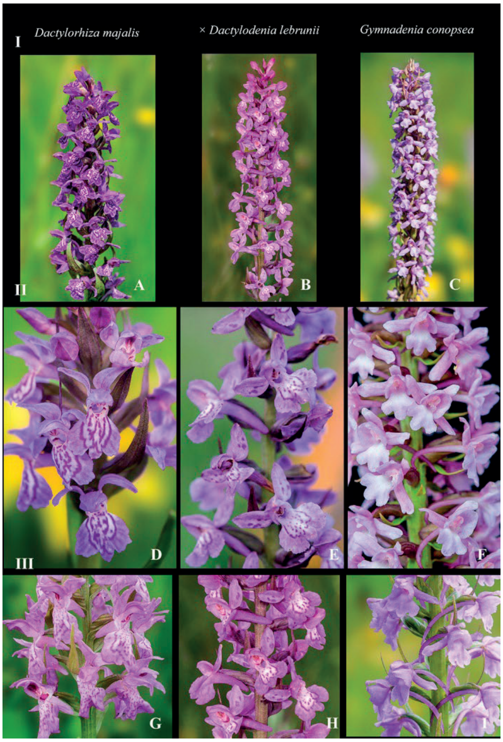


Figure 9. × *Dactylodenia lebrunii* (E.G. Camus) Peitz, 1972
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza majalis*; **B., E., H.** - × *Dactylodenia lebrunii*; **C., F., I.** - *Gymnadenia conopsea*. Photos B., H. © Lori Balogh; Photos A., C.-G., I. © Nora E. Anghelescu, 2 June 2023, Vlăhița, Harghita County

Hybrid formula: *D. majalis* × *G. conopsea*. **Discovered:** 2 June 2019, Lori & Mihaela Balogh. **Locus classicus:** Vlăhița, Harghita County. **Flowering time:** May-June. **Native to:** Austria, Czechoslovakia, France, Germany, Poland, Sweden, Switzerland Romania. **Description:** Stem (epigeal), 23(27) cm, erect, vivid green, mildly purple-tinged at tip, resembling *D. majalis*. 1 basal leaf, 2-3 cauline leaves, purple-pigmented, arched, elongate-acuminate resembling *D. majalis*. Inflorescence dense raceme, very floriferous, cylindrical-elongated, resembling *G. conopsea*. Flowers pinkish-purple, medium-sized, 5-8 mm diameter, resembling *D. majalis*. Lateral sepals roundish-ovoidal, widespread (resembling *G. conopsea*), dorsal sepal and lateral petals forming a loose hood, resembling *D. majalis*. Labellum flat, wide, three-lobed, lateral lobes scalloped (resembling *G. conopsea*), median lobe narrower, pointed, marked with dark purple loops and stripes resembling *D. majalis*. Spur equal/longer than the ovary, pointed downwards, resembling *G. conopsea*. **Population counts:** single individual. **Herbarium voucher specimen:** deposited at the Herbarium Botanical Garden Bucharest: ***BUC 410365.*** **Proposed conservation status:** Endangered (EN).

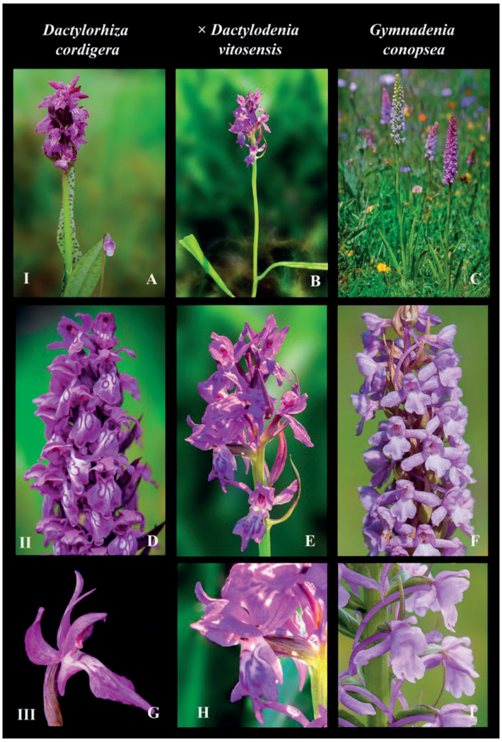


Figure 10. × *Dactylodenia* cf. *vitosensis* Jagiello, 1988
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza cordigera;* **B., E., H.** - × *Dactylodenia* cf. *vitosensis;* **C., F., I.** - *Gymnadenia conopsea*. Photos B., E., H. © Remus Dulugeac, 26 May 2018, Argeș County; Photos A., C., D., F., G., I. © Nora E. Anghelescu, 10 July 2023, Bucegi Natural Park

Hybrid formula: *D. cordigera* × *G. conopsea*. **Discovered:** 26 May 2018, Remus Dulugeac. **Locus classicus:** Argeș County. **Flowering time:** May-June. **Native to:** Bulgaria, Romania. **Description:** Stem (epigeal), 14(16) cm, erect, vivid green, spindly, resembling *G. conopsea*. 1-2 basal and 1 cauline leaves, vivid-green, non-pigmented, narrow-lanceolate, acuminate, widest in the middle, resembling *G. conopsea*. Inflorescence short, dense raceme, less floriferous, flowers purple, small sized, resembling *D. cordigera*. Lateral sepals elongated, reflexed to spreading, resembling *D. cordigera*. Dorsal sepal and lateral petals forming a loose hood, resembling *D. cordigera*. Labellum three-lobed, very similar in shape and labellar markings to *D. cordigera*. Spur rather more elongated than that of a typical *D. cordigera*, with a mild resemblance to *G. conopsea*. This unique individual is still under study. Its full identification is still to be confirmed, since, except for the leaves and possibly the lengths of the spur, this hybrid resembles more a *D. cordigera* individual with abnormally non-pigmented, elongated leaves. **Population counts:** single individual. **Proposed conservation status:** Endangered (EN).

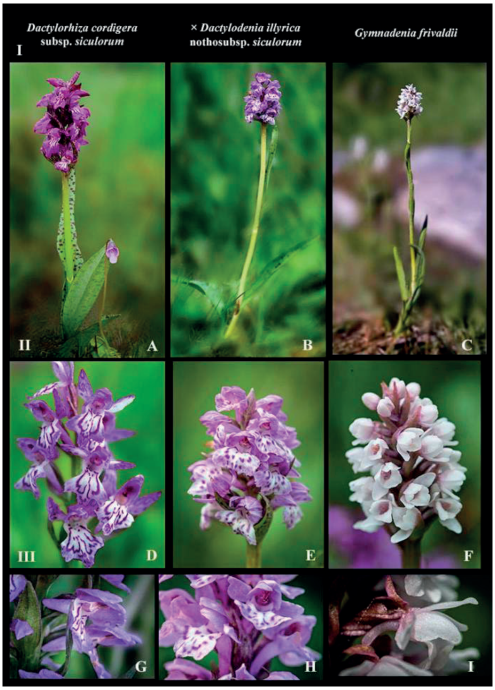


Figure 11. × *Dactylodenia illyrica* nothosubsp. *siculorum* L.Balogh & Mih.Balogh, N.Anghelescu, N. Kigyossy, 2023 nothosubsp. *nov*.
I. Full plants in their natural habitats; **II.** Inflorescences details**; III.** Individual flower detail; **A., D., G.** - *Dactylorhiza cordigera* subsp. *siculorum***; B., E., H.** - × *Dactylodenia illyrica* nothosubsp. *siculorum*; **C., F., I.** - *Gymnadenia frivaldii*. Photos © Nora E. Anghelescu, 9 June 2023, Harghita Mădăraş, Harghita

Hybrid formula: *D. cordigera* subsp. *siculorum* × *G. frivaldii***. Discovered:** 20 june 2022, Lori & Mihaela Balogh. **Locus classicus:** Harghita Mădăraş, ROSCI00090. **Flowering time:** June-July. **Native to:** Romania, endemic, ***nothosubsp. nov*. Description:** Stem (epigeal), 10(12) cm, erect, vivid green, spindly, resembling *G. frivaldii*. 1 basal and 1-2 cauline leaves, vivid green, purple-pigmented with minute roundish-elongated, acuminate, purple-brownish maculae, wider toward the middle, resembling *D. cordigera* subsp. *siculorum*. Inflorescence short, dense raceme, resembling *G. frivaldii* in shape and size. Flowers small, pinkish, with flat labellum, wider than long, whitish in the middle, pink-purple towards the margins, marked with dark-purple dots, resembling *D. cordigera* subsp. *siculorum*. Lateral sepals spreading, resembling *G. frivaldii*. Hood flat and tight resembling *G. frivaldii*. Spur elongated-cylindrical, purplish, resembling *D. cordigera* subsp. *siculorum*. Flower bracts, wide, triangular, keeled, marked with purple-black maculae resembling *D. cordigera* subsp. *siculorum*. **Population counts: *Nothopopulation of two hybrids*. Herbarium voucher specimen:** deposited at the Herbarium Botanical Garden Bucharest: ***BUC 410364*. Proposed conservation status:** Endangered (EN).

Figure 12. × *Pseudadenia vitosana* (H. Baumann) O. Gerbaud & W. Schmid, 1999
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Gymnadenia frivaldii*; **B., E., H.** - × *Pseudadenia vitosana*; **C., F., I.** - *Pseudorchis albida* subsp. *tricuspis*. Photos © Nora E. Anghelescu, 1 July 2023, Harghita Mădăraş, Harghita County

Hybrid formula: *G. frivaldii* × *P. albida* subsp. *tricuspis*
Discovered: 20 Iunie 2022, Lori & Mihaela Balogh. **Locus classicus:** Harghita Mădăraş, ROSCI00090. **Flowering time:** June-July. **Native to:** Albania, Bulgaria, Yugoslavia, Romania. **Description:** Stem

(epigeal), 15-30 cm, erect, vivid green, spindly, resembling *P. albida* subsp. *tricuspis*. 1 basal and 4-6 cauline leaves, vivid green, keeled, wider towards the upper half, resembling *P. albida* subsp. *tricuspis*. Upper cauline leaves sheathing the stem, resembling *G. frivaldii*. Inflorescence elongated-cylindrical, floriferous raceme, resembling *P. albida* subsp. *tricuspis*. Flowers small, whitish-yellowish, with flat, three lobed-labella, with lobes near equal, resembling *P. albida* subsp. *tricuspis*. Lateral sepals spreading horizontally, the hood flat and tight resembling *G. frivaldii*. Spur intermediate, whitish, nectariferous. **Population counts:** ***Nothopopulation of nine hybrids*.** **Herbarium voucher specimen:** deposited at the Herbarium Botanical Garden Bucharest: ***BUC 410374*.** **Proposed conservation status:** Endangered (EN).

Figure 13. × *Pseudadenia schweinfurthii* (Hegelm. ex A. Kern.) P.F. Hunt, 1971
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Gymnadenia conopsea*; **B., E., H.** - × *Pseudadenia schweinfurthii*; **C., F., I.** - *Pseudorchis albida* subsp. *tricuspis*. Photos © Nora E. Anghelescu, 1 July 2023, Harghita Mădăraş, Harghita County

Hybrid formula: *G. conopsea* × *P. albida* susp. *tricuspis*

Discovered: 20 June 2023, Nora E. Anghelescu. **Locus classicus:** Harghita Mădăraş, ROSCI00090. **Flowering time:** June-July. **Native to:** Austria, Czechoslovakia, Germany, Norway, Sweden, Switzerland, Romania. **Description:** Stem (epigeal), 25-35 cm, erect, vivid green, spindly, resembling *G. conopsea*. 1 basal and 3-4 cauline leaves, vivid-green, keeled, narrower, acuminate, resembling *G. conopsea*. Inflorescence cylindrical, floriferous raceme, resembling *P. albida* subsp. *tricuspis*. Flowers small, yellowish-pink (resembling *G. conopsea*), with flat, three lobed-labella, with lobes nearly equal, tooth-like, resembling *P. albida* subsp. *tricuspis*. Lateral sepals spreading horizontally, the hood flat and tight resembling *G. conopsea*. Spur intermediate, whitish, nectariferous. **Population counts:** single individual. **Herbarium voucher specimen:** deposited at the Herbarium of Botanical Garden Bucharest: ***BUC 410373*.** **Proposed conservation status:** Endangered (EN).

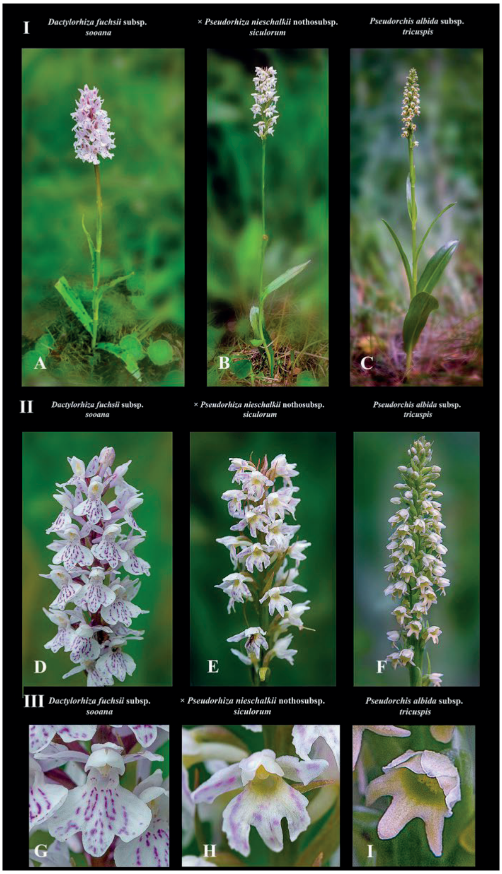


Figure 14. × *Pseudorhiza nieschalkii* nothosubsp. *siculorum* H. Kertész & N. Anghelescu, 2020
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail**; A., D., G.** - *Dactylorhiza fuchsii subsp. sooana*; **B., E., H.** - × *Pseudorhiza nieschalkii* nothosubsp. *siculorum*; **C., F., I.** - *Pseudorchis albida* subsp. *tricuspis*. Photos © Nora E. Anghelescu, 3 July 2020, Harghita Mădăraş.

Hybrid formula: *D. fuchsii* subsp. *sooana* × *P. albida* subsp. *tricuspis*. **Discovered:** 30 June 2020, Hajnalka Kertész. **Locus classicus:** Harghita Mădăraş, ROSCI00090. **Flowering time:** June-July. **Native to: Romania, *endemic*.** **Description:** Stem (epigeal), 28(29) cm, erect, vivid green, spindly, resembling *P. albida* subsp. *tricuspis*. 1 basal leaf, 2-3 cauline, unspotted leaves, resembling *P. albida* subsp. *tricuspis*. Upper cauline leaf bract-like, resembling *D. fuchsii* subsp. *sooana.* Inflorescence short, dense raceme, resembling *D. fuchsii* subsp. *sooana* (De Angelli & Anghelescu, 2020). Flowers small, yellowish-white, with flat, three-lobed labellum with three equal lobes (length and width), resembling *P. albida* subsp. *tricuspis*. Lateral sepals spread laterally, white with pinkish dots and stripes, resembling *D. fuchsii* subsp. *sooana.* Labellum and lateral sepals are marked with purple-pinkish dots and lines, resembling *D. fuchsii* subsp. *sooana.* The white background of the tepals and labellum resembles *D. fuchsii* subsp. *sooana.* The hood is tight, formed of dorsal sepal and lateral petals resembling *D. fuchsii subsp. sooana* (Anghelescu *et al.,* 2021a). **Population counts:** single individual. **Herbarium voucher specimen:** deposited at the Herbarium of the Botanical Garden Bucharest: ***BUC 410375***. **Proposed conservation status:** Endangered (EN).

CONCLUSIONS

The genetic diversity within a species or population is the outcome of long-term evolutionary processes and forms the foundation for its survival and adaptability (Soltis & Soltis, 2009). Various species exhibit varying degrees of genetic diversity and variation, allowing them to adapt to various environmental conditions (Wu et al., 2023). The formation of new species (a phenomenon known as speciation), is primarily driven by the development of the reproductive isolating barriers that gradually diminish and ultimately prevent significant gene flow between distinct lineages (Christie et al., 2022). There is a general consensus that the speed and manner of speciation are significantly influenced by the type of isolating barriers (Coyne & Orr, 2004). In plants, these barriers are most conveniently categorized as either premating (prezygotic) or postmating (involving both prezygotic and postzygotic) isolation mechanisms (Grant, 1981; Snow, 1994).

Hybridization was demonstrated to represent a dynamic process in evolution that can lead to genetic diversity, adaptation, and the formation of new nothospecies. Novel genetic lineages occur in many, specific hybrid zones implying that hybrids are fertile and can coexist with the parental species.

These results, together with the prediction of suitable conditions for the future occurrence of these hybrid zones, highlight the importance of conserving these geographic areas as sources of novel taxonomic entities. The high frequency of hybrids suggests low efficiency of reproductive barriers in genera such as *Anacamptis* and *Ophrys* or between genera such as *Dactylorhiza, Gymnadenia* and *Pseudorchis*. Given that a high number of viable and fertile hybrid seeds were produced, hybridization generated several genetic novelties in all hybrid zones.

Our studies highlight the complex relationships between different species and their genetic material over geological time scales, contributing to the rich tapestry of life on Earth.

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ANALYSIS OF THE DENDROFLORISTIC COMPOSITION OF URBAN STREET TREE PLANTINGS IN SOFIA

Svetlana ANISIMOVA

University of Forestry, 10 Kliment Okhridsky Blvd, 1797, Sofia, Bulgaria

Corresponding author email: sanisimova@ltu.bg

Abstract

Street landscaping is that part of the urban green infrastructure that is crucial for urban heat island mitigation, climate change adaptation and biodiversity protection. Moreover, it provides city dwellers with various ecosystem services and daily accessible public greenspace. The aim of this research is to assess the street tree diversity of the city of Sofia. A total of 10,011 street tree specimens were inventoried. The field studies were conducted from 2021 to 2022, according to the route method. The selection of the sample streets for the survey was made based on the following criteria: street tree plantings covered all street classes of the primary and secondary street network and the variety of possible orientations and street canyon geometries; street tree plantings were located in different administrative territorial units of Sofia Municipality and the survey covered the entire length of the streets. In order to make an approximate assessment of the age of the specimens along the surveyed streets, DBH of each street tree stem was also collected and classified into categories. The established species composition in the surveyed streets consists of 55 taxa (species and cultivars). The most commonly used tree species in the new street plantings of the whole city street network was Platanus x *acerifolia (15.09%), while the most prevalent genera was Fraxinus (19.58%). One of the important findings in the analysis of the dendrofloristic composition was the low species diversity at street level. In most of the streets the number of species participating with more than 10% is 2-3. The analysis of the ratio of native to non-native (incl. cultivated varieties) street tree species showed that the non-native species and infraspecific taxa accounted for 57.32% of the total number of specimens. The results of the study can provide general guidelines for sustainable street plantings planning and design incl. selection of dendrofloristic composition for diverse street tree populations.*

Key words: *biodiversity, street trees, landscape design.*

INTRODUCTION

Street landscaping is that part of the urban green infrastructure that is crucial for urban heat island mitigation, climate change adaptation and biodiversity protection. Moreover, it provides city dwellers with various ecosystem services and daily accessible public greenspace near their home and workplace (Salmond et al., 2016; Ekkel & de Vries, 2017; Endreny, 2018; Marselle et al., 2020).

Urban streets are an inhospitable environment for tree growth (Paganová and Jureková, 2012). Some of the abiotic and biotic stress factors associated with street planting locations include anthropogenic compacted soils, inadequate soil volume and quality, impervious surfaces, overheating of the root zone, water deficiency, soil salinity caused by the accumulation of road de-icing salts, artificial lighting, air and soil pollution, urban heat island and street canyon effect, improper pruning, mechanical damage, etc. These harsh conditions not only negatively impact tree growth and mortality, but also reduce the list of appropriate street tree species and genera (Cowett & Bassuk, 2017).

The design approach adopted in Europe in the sixteenth century (Couch, 1992) to achieve a uniform silhouette and foliage texture along street, avenue, boulevard profiles has led to many even-aged monoculture street plantations. However, the desired visual uniformity in street tree planting may be accomplished by using diverse taxa which exhibit sufficient similarities according to citizens' perceptions (Vogt et al., 2017).

Historically, the uniform single-species formal planting scheme has been appreciated both from the design point of view and in terms of simplified management (Roman & Eisenman, 2022). However, the dendrofloristic and genetic diversity of tree populations has been associated with enhanced resilience to climate change (Lohr et al., 2016) and pests and pathogens (Laçan & McBride, 2008). The monocultures in urban green infrastructure pose a risk of large-scale pest and disease outbreaks (Santamour, 1990). In this context, street tree diversity assessment has been considered part of sustainable urban tree population management (Raupp et al., 2006; Sjöman & Östberg, 2019).

Santamour (1990) suggested the so-called 10-20-30 rule for ensuring diversity within a municipality's street tree population. The author proposed that no tree community should comprise: (1) more than 10% of any particular tree species; (2) more than 20% of any one tree genus, and more than 30% of any single family. Moreover, Santamour emphasized the importance of even distributions of street tree taxa (families, genera, species, cultivated varieties, clones) in urban street tree populations in order to achieve diversity at spatial and biological levels.

Some forest researchers dismissed Santamour's rule, because there is little empirical evidence to support these reference indices (Kendal et al., 2014) and proposed stricter rules for managing diversity. Bassuk et al. (2009) recommended that any street tree species should be limited to between 5% and 10% of the whole street tree population, while Ball (2015) suggested that any street tree genus should be limited to 5% of the street tree population.

Another important public health aspect related to increased species biodiversity and distribution evenness in urban green infrastructure is the avoidance of exposure to large, concentrated sources of monospecific allergenic pollen, one of the ecosystem disservices associated with urban street trees (Cariñanos & Casares-Porcel, 2011).

Additionally, these linear elements of green infrastructure, providing interconnection between green spaces, have been of a great importance to urban wildlife, and to avifauna in particular (Lepczyk et al., 2017). The value of indigenous species and selected exotic woody species utilized in street trees plantings as habitats and food resources for birds is high (Narango et al., 2017; Wood & Esaian, 2020).

The history of street tree plantings in the city of Sofia started after the Liberation. After 1879, with urban development, the first avenue trees were planted along the new city streets and boulevards. The intensive development of street landscaping began in 1880-1883, after the adoption of the first Master plan of Sofia. The main species in the streetscape in that period were *Robinia pseudoacacia* L. and *Aesculus hippocastanum* L. By the end of 1941, for a 60-year period, more than 30,000 street tree specimens from the following taxa were planted: *Robinia pseudoacacia* 'Umbraculifera', *Acer* spp., *Platanus* spp., *Betula pendula* Roth, *Fraxinus* spp., *Populus* spp. among others. Later, in the period 1945-1965, *Quercus rubra* L. and *Tilia* spp. specimens were used along some boulevards (Kuleliev, 1994).

A more recent study (Delkov & Gateva, 2004) of the dendrological structure of Sofia street plantings determined *Fraxinus* spp. (27.9%), *Aesculus hyppocastanum* L. (15.5%), *Populus* spp. (13.5%), *Robinia pseudoacacia* L. (9.2%), *Acer* spp. (8.9%), *Tilia* spp. (8.9%), *Betula pendula* Roth. (7.6%), and *Quercus rubra* L. (1.4%) as the most widespread taxa.

Currently, Sofia Municipality is developing an interactive map and register of green areas and mapped vegetation (https://ropkr.sofia.bg/) for the trees in its green infrastructure, incl. those lining streets, but it has not been completed yet.

The aim of this research is to assess the street tree diversity of the city of Sofia.

Collecting a street tree inventory data is essential for the management of this urban green infrastructure element. A total inventory (census) and mapping of the street trees specimens provide comprehensive data on the dendrofloristic composition, spatial distribution, prevailing DBH/ age classes, health status and maintenance issues. Moreover, sampling of street tree populations also provides efficient general information on street tree population characteristics (Smiley & Baker, 1988; Nowak et al., 2015).

MATERIALS AND METHODS

The street tree diversity has been assessed based on the data collected from sample street tree on-site inventory of Sofia street tree populations.

The field studies to establish the dendrofloristic composition were conducted from 2021 to 2022, according to the route method. The selection of the sample streets for the survey was made based on the following criteria: street tree plantings should cover all street classes of the primary and secondary street network and the variety of possible orientations and street canyon geometries; street tree plantings should be located in different administrative territorial units of Sofia Municipality and the survey should cover the entire length of the streets.

In order to make an approximate assessment of the age of the specimens along the surveyed streets, the dendrometric indicator - diameter at breast height (DBH) of each street tree stem was also collected and classified into categories.

The metrics employed to assess street tree diversity were: frequency distribution and Simpson's Diversity Index**.**

The relative abundance/distribution evenness of street tree taxa (species and genera) were calculated as a percentage at different levels (single street tree population, street tree population of different street classes, street tree population of the whole city).

Simpson's Index of Diversity (Simpson, 1949) considers the population size and species richness (the number of species in the population). The Inverse Diversity Index (1/D) was adopted for measuring species diversity. A higher value (near to 1) represents greater diversity, and a lower value (close to 0) indicates lower diversity.

An analysis of the ratio of native to non-native tree species (incl. cultivated varieties) and of a prevailing indigenous species was performed.

RESULTS AND DISCUSSIONS

General characteristics of Sofia street network

The street tree diversity of street tree populartions along 46 streets in Sofia was assessed, both within the Central city part and in the territory outside it (Figure 1). A total of 10,011 specimens were inventoried, 3,258 - along II class streets, 3,895 - along III class streets, 1,269 - along IV class streets and 1,589 - along V class streets.

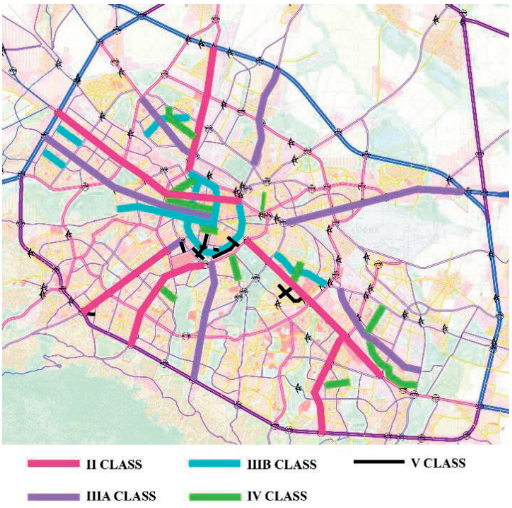

Figure 1. Surveyed streets in the Sofia street network

The focus of the research was the streets of the primary street network (II, III, IV class streets). The survey covered 6 II class streets, 8 III-A class streets, 10 III-B class streets, 9 IV class streets. They are the main communication and transport system of the urbanized territories and connect the different administrative territorial units of Sofia Municipality with each other, as well as with the republican and municipal road network. In connection with their function, these are streets with heavy vehicular and pedestrian traffic and with unfavorable conditions for the development of woody vegetation compared to the streets of the secondary street network.

The streets of the secondary street network lead and distribute the traffic from the primary street network to the urbanized units and serve individual properties. A total of 13 V class streets were surveyed.

The surveyed trees were mainly ones planted in sidewalk cutouts and rarely in road median strips or curb strips along all street classes.

Dendrofloristic composition of Sofia street plantings

The results from the inventory of the dendroflo-ristic composition of urban street tree plantings in Sofia showed that the total number of taxa was 55 in terms of species and infraspecific taxa (cultivated varieties), belonging to 19 families and 30 genera. This list includes a lot of single specimens of a taxa, while the diversity along streets in most cases was dominated by 18 taxa (Figure 2). The ones with a relative abundance greater than 5% were *Populus* x *euroamericana* (5.59%), *Fraxinus angustifolia* Vahl. (5.70%), *Tilia tomentosa* Moench. (5.85%), *Quercus rubra* L. (6.31%), *Fraxinus excelsior* L. (incl. 'Globosa') (8.36%), *Aesculus hippocastanum* L. (9.73%). The taxa with a relative abundance greater than 10%, which exceeded Santamour's 10% rule for species, were *Acer platanoides* L. (incl. 'Globosum', 'Crimson King'/'Royal Red') (11.74%) and *Platanus* x *acerifolia* (Aiton) Willd. (15.09%).

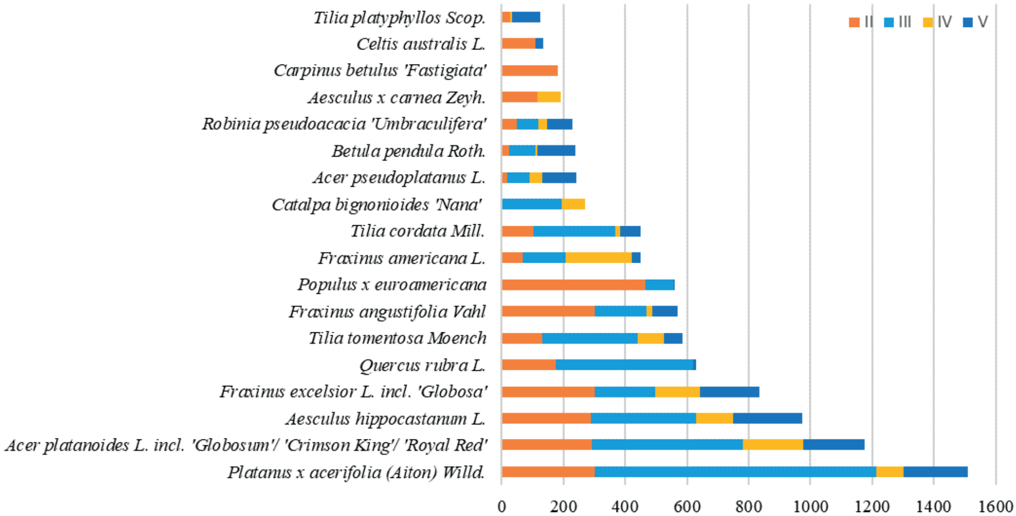

Figure 2. Relative abundance of street tree species in Sofia

These results support the worldwide tendency of *P.* x *acerifolia* to dominate as a street tree.

The European pilot tree survey has shown that species from the genera *Platanus* L., *Aesculus* L., *Acer* L., *Tilia* L. comprise 50-70% of all street trees planted (Pauleit, 2003).

Platanus x *acerifolia* was the most abundant street tree species in Amsterdam, Bologna, Buenos Aires, Melbourne, and Paris, while *Acer* spp. were the most dominant species in the streets of Cambridge, Oslo, and Vancouver (Galle et al., 2021).

The common species for street tree plantings in Bucharest were *F. excelsior, P.* x *acerifolia, A. hippocastanum*, *Tilia* spp., among others. Moreover, *P.* x *acerifolia* was found to be a dominant species (up to 88%) for some alignments (Dobrescu & Fabian, 2017). A recent survey of two New Belgrade boulevards showed that *P.* x *acerifolia* specimens accounted for 30.14% and 79.13%, respectively, which makes it the prevailing taxa among street trees (Milutinović et al., 2022).

In recent years the number of *Platanus* trees in the city of Poznan has shown a noticeable increase with a total number of over 1,500 specimens (Nowak et al., 2012). This species prevails in street tree plantings in northeastern US cities (Roman & Eisenman, 2022).

Platanus x *acerifolia* demonstrates high Environmental Stress Tolerance (EST) in terms of resilience to water stress and toxic ambient urban pollutants (Tiwary et al., 2016). On the other hand, *Platanus* spp. have been proved to be one of the main urban emitters of allergenic pollen (Cariñanos et al., 2020; Magyar et al.,

2022) and Biogenic Volatile Organic Compounds (BVOCs) (Xiaoshan et al., 2000; Curtis et al., 2014; Jing et al., 2020).

In relation to the aforementioned disservices, its further planting in public urban green areas should be restricted. Moreover, Georgieva et al. (2023), assessing the health status of this species in urban green areas of Sofia, noted the impact of biotic factors, pests and fungal pathogen infections on the performance of *Platanus* specimens. The authors warned about the potential threat posed by the pathogen *Ceratocystis platani* that had a destructive effect on the green infrastructure of the neighboring Greece and Turkey, causing the widespread mortality of plane trees.

The most common indigenous species in the streetscape of Sofia was *A. hippocastanum*, which is considered to be a symbol of the city of Sofia. However, the specimens cultivated as urban street trees had not only reduced vitality and longevity, but also low ornamental performance. Moreover, the health status of 22% of the investigated specimens made them potentially hazardous for city dwellers. (Pencheva & Anisimova, 2016).

The inventory data on street tree species composition revealed that the species that exceeded Santamour's 10% rule were: for II street class - *P.* x *euroamericana* (14.33%), while *F. angustifolia* (9.24%) and *P.* x *acerifolia* (9.24%) were close to the benchmark; for III street class - *P.* x *acerifolia* (23.42%) and *Q. rubra* (11.43%); for IV street class – *F. americana* L. (15.78%) and *A. platanoides* 'Globosum' (10.96%); for V street class - *A. hippocastanum* (14.02%), *P.* x *acerifolia* (13.07%), *A. platanoides* 'Globosum' (11.19%).

The inventory data on street tree genus composition revealed that the genera that exceeded Santamour's 20% benchmark were: for II street class *Fraxinus* (21.03%), for III street class - *Platanus* (23.52%); for IV street class - *Fraxinus* (33.68%), *Acer* (19.48%) was close to the benchmark; for V street class - *Fraxinus* (21.68%), *Acer* (19.92%) was close to the benchmark.

The Inverse Simpson's Diversity Index (1/SDI) was calculated for the whole street population as an index that takes into account both species richness and the evenness of the species present. The high species diversity of Sofia street trees (0.94) was attributed mainly to the greater number of less abundant species rather than to their more even distributions. However, it was found that just 2.49 ± 0.99 number species with relative abundance greater than 10% comprise 84.46 ± 13.14% of the total street tree population at street level.

Among all street tree genera, *Fraxinus* was found to be the most prevalent in the whole city street network (19.58%), followed by *Platanus* (15.34%), *Acer* (14.38%), *Aesculus* (11.70%), *Tilia* (11.60%) (Figure 3). These five genera comprised 72.62% of the total street tree population. *Fraxinus*' relative abundance was close to Santamour's benchmark at genus level - 20%.

In comparison, *Tilia* was found to be the most common genus (21.09%) of the whole tree population in ten major Nordic cities, followed by *Acer* (12.34%), *Sorbus* (10.41%) and *Betula* (10.22%) (Sjöman & Östberg, 2019).

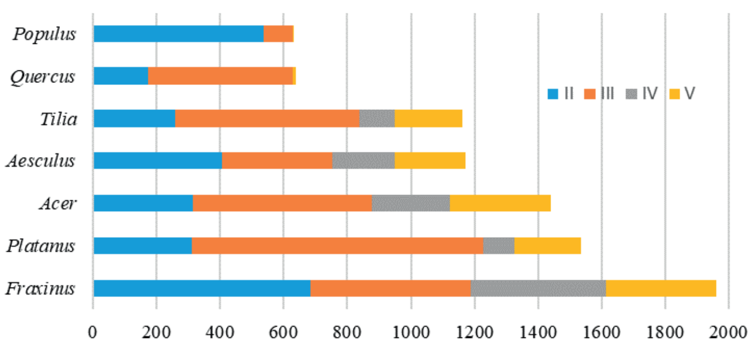

Figure 3. Relative abundance of the most common genera in Sofia street tree plantings

The analysis of family composition revealed that none of the families surpassed Santamour's 30% rule for family level - Sapindaceae (26.09%), Oleaceae (19.58%), Platanceae (15.34%), Malvaceae (11.6%).

The age structure of the street tree population of the surveyed streets was assessed using the dendrometric indicator - diameter at breast height (DBH) (Figure 4).

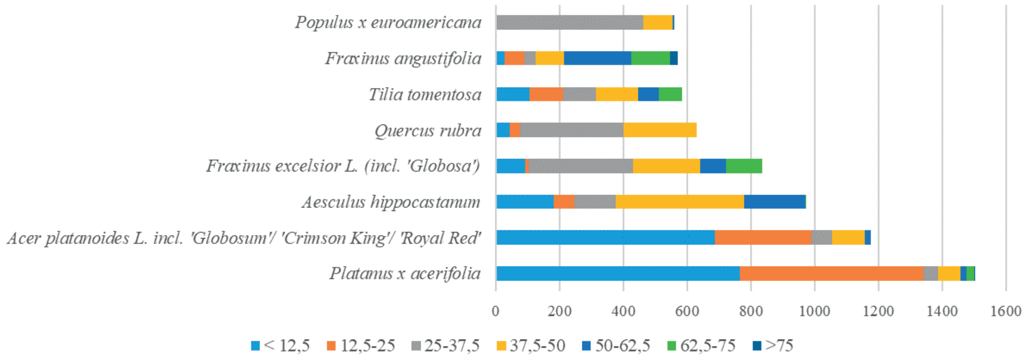

Figure 4. Structure by categories of diameter classes for the main species

The results show that about 1/2 of the specimens were up to 25 cm in diameter, and nearly 15% were over 50 cm in diameter, which is an indicator of aging and a cause for urgent measures for their phased replacement. The analysis of DBH indicated the dominant presence of young *P.* x *acerifolia* and *Acer platanoides* 'Globosum' specimens (DBH < 25).

The key to increasing diversity and making a significant structural change in municipal street tree dendrofloristic composition, respectively, is a long-term strategy of replacing more dominant species and genera that have reached senior stage with less prevalent and appropriate species and genera. Thus, the transition to greater diversity may be accomplished more quickly for municipalities where the street tree population is in decline than in municipalities with younger street tree plantings (Cowett & Bassuk, 2017). Biodiversification at different taxonomic ranks could be achieved by planting underutilized native tree species (Hilbert et al., 2022).

As part of the dendrofloristic characteristics, the analysis of the ratio of native to non-native (incl. cultivated varieties) street tree species showed that the non-native species and infraspecific taxa accounted for 57.32% of the total number of specimens (Figure 5).

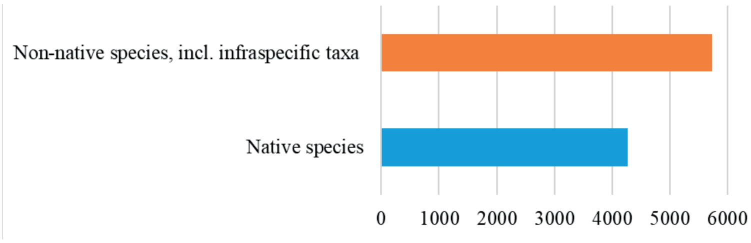

Figure 5. Relative abundance of native and non-native species, incl. infraspecific taxa

A concerning trend towards the large scale use of exotic species and cultivars in the green infrastructure was reported in many studies (Pysek et al., 2009; Morgenroth et al., 2016; Anisimova, 2018; Useni Sikuzani et al., 2022).

CONCLUSIONS

The results indicate a relatively low species diversity in the current city street populations at street level. The most commonly used tree species in the new street plantings of the whole city street network was *Platanus* x *acerifolia,* while the most prevalent genera was *Fraxinus*.

A database should be developed with a complete street tree inventory, which has to be updated regularly in order to contain comprehensive data on streetscape species composition, age structure and spatial distribution, health status, establishment of young trees, essential information for street tree planning and management. Long-term observations could track and evaluate changes in the street tree population in the context of climate change.

Official guidelines/standards on the management and maintenance of street tree plantings, incl. monitoring, should be developed and adopted by Sofia Municipality.

The concept of sustainable development of urban green infrastructure should include not only the spatial distribution of street plantings, but should also define the street tree selection criterion and species composition in order to ensure adequate street tree diversity at different levels (at street level, administrative territorial level and city level).

The results from such inventories should be taken into consideration by local nurseries in order to organize production/import and to provide the municipality with semi-mature, properly trained, standard street trees from diverse and less prevalent species and genera.

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USING THE PRINCIPLES OF THERAPEUTIC GARDENS IN THE PROPOSED REDEVELOPMENT OF THE GREEN SPACE OF THE "PROF. DR. NICOLAE OBLU" HOSPITAL IN IASI

Codrina GRECU, Mirela COJOCARIU, Clara-Teodora BALAN, Doina-Mira DASCĂLU

University of Life Sciences “Ion Ionescu de la Brad” of Iasi, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: mirela_c@uaiasi.ro

Abstract

The paper presents a study on therapeutic gardens with an example of applying their composition rules within a landscaping project proposal for the green space of the Hospital "Prof. Dr. Nicolae Oblu" in Iasi. These therapeutic gardens have been used over the years as specially landscaped outdoor spaces near hospitals or in the inner courtyards of various rehabilitation or treatment centers. These gardens' design and construction principles help improve and heal patients' ailments more quickly and contribute to the well-being of all institution staff and visitors. The same rules and principles underlie the garden design concept of the Iasi Clinical Neurological Hospital, which, through this redesign proposal, has been given the possible status of a public garden with a warm and welcoming atmosphere, which also meets the criteria specific to therapeutic gardens.

Key words*: therapeutic gardens, hospitals green areas landscaping, landscape design principles.*

INTRODUCTION

Historical documents indicate that designing the green spaces associated with healthcare facilities was essential to making patients feel comfortable and heal quickly.

The notion of a healing space has its roots in ancient Greece, where there were temples such as the Sanctuary of Epidaurus, where sick people went in the hope of having dreams in which cures for their illnesses was revealed.

At the same time, Egypt's great gardens were also built to escape the everyday external environment and induce healing relief.

Since the Middle Ages, therapeutic gardens have been places of recuperation, restoring mind, soul and body. Monastery hospitals used indoor gardens as areas for therapy and healing. The Zaragossa Hospital in Spain, built in 1409, is an example used as an inspiration for landscape architects of the time, especially regarding how patients could interact. Patients were encouraged to go out into the hospital courtyard and communicate with each other throughout the day in the open air. Non-disabled patients were involved in activities such as gardening.

The European Romanticism movement of the 18th century brought about essential changes in the layout of hospital units and grounds. The theory linking successful medical therapy to a natural environment around hospitals was revived. Cooper Marcus noted that in the 19th century, Dorothea Linde Dix (1802-1887) was the first to take an interest in modifying patient therapy methods and, by extension, the hospital environment. She proposed to the American Legislature certain basic principles regarding the arrangement of areas in these institutions.

However, in the 20th century, advances in medicine, urbanization, technological developments, and other economic forces gradually led to neglecting of hospital green areas, and many parking lots replaced them.

In 1984, a study by the American psychologist Roger Ulrich brought the importance of hospital gardens back to the fore. Ulrich demonstrated that patients who had a view of the hospital's outdoor green spaces in their rooms recovered faster after surgery and spent less time in the hospital than those who did not. Since the 1990s, the idea of healing gardens has regained interest, and various research in sustainable landscaping began to appear (Vapaa, 2002).

Horticultural therapy developed from occupational therapy in the USA in the 1950s. Subsequently, many hospitals in Europe added so-called Horticultural Therapy to their therapeutic programs to keep patients' minds occupied and lead to creative action. Nowadays, horticultural activity therapy is becoming increasingly widespread, as Buru et al. (2022) point out in their paper on its influence on the quality of human life. Other research has shown that some medicinal plants in urban gardens can improve air and soil quality by absorbing atmospheric or soil pollutants, which can be an added advantage for a therapeutic garden in a hospital (Hangan et al., 2020).

The benefits of patient exposure to a therapeutically landscaped hospital garden are listed and described by Professor Emeritus P.D. Relf (2019) in his paper on institutional healthcare gardens. Both Relf and other researchers have described in their work the benefits observed in users of these types of therapeutic gardens as follows:

- Physiological benefits: lower pulse rate and muscle and blood pressure, lower consumption of pain relievers, and faster recovery after surgery or other types of trauma (Gerlach et al., 1998);
- Psychological benefits: patient compliance with therapy, security, reduced stress, aiding sensory development, employee job satisfaction (Ulrich, 1999; Cooper Marcus and Barnes, 1995; Ulrich, 1992);
- Social benefits: encourage social contact and interaction and allow visitors to enjoy nature (Dascălu and Cojocariu, 2016; Cooper Marcus and Barnes, 1995).

As a result of the information previously described, the present work aims to design green spaces with therapeutic value on the premises of the Emergency Clinical Hospital "Prof. Dr. Nicolae Oblu" in Iasi. The main objective of this proposal is to enhance medical recovery by increasing the quality of the hospital environment and ensuring a higher degree of comfort for patients, medical staff, and visitors.

MATERIALS AND METHODS

When designing a space for a healthcare facility, the focus should be on location, accessibility, patient requirements and preferences, and design elements to be included. The garden should have opportunities for mobility and exercise, present a choice between social and solitary spaces, and facilitate healthy recreation and direct or indirect interaction with nature.

Variety criterion

To enable the most significant therapeutic benefits, the therapeutic garden should have a variety of spaces for groups and solitary individuals, giving the patients choice and an increased sense of control, leading to lower stress levels. Equally, variety should apply to vegetation. The design should include seasonal flowering species, plants that attract small and safe wildlife (birds, squirrels, butterflies), and species with different foliage or grasses that move under the influence of air currents. Natural features such as trees, shrubs, flowers, and water feature lower stress levels. Other activities, such as working with plants and gardening, can also provide users with relaxing moments in the garden. Hard landscaping should be kept to a minimum, and plant materials should dominate the space. Hospital spaces should minimize negative factors such as urban noise, smoke, and artificial lighting and enhance the therapeutic value of natural light and sound. Gardens that appeal to different senses are often ideal, although strongly scented flowers and other odors should be avoided for chemotherapy patients. In general, it is advisable to have open views of the sky and clouds and, if possible, the horizon. Pools with fish or water lilies or moving water that can be seen and heard should be introduced.

Accessibility criterion

Gardens can be attractively designed and landscaped, but people need to know that they exist and are easily accessible via entrances and pathways. This is an essential requirement within the hospital and its external environment. It is also essential that they are usable by all users, regardless of age or different mobility impairments, and at the same time only facilitate certain activities.

Visibility criterion

The more visible a garden is, and the more people are aware of it, the more they will prefer its activity areas. At least one outdoor area should be visible, or its location should be indicated from the main entrance. Patients' rooms should have a view of the garden to enjoy it even if they cannot visit it.

Feelings of control and security criteria

Patients often feel physically and psychologically vulnerable in hospitals, so providing them with a sense of security is advisable. Research shows that a lack of control can induce or worsen depression, passivity, increased blood pressure, and decreased immune system function. A sense of control in the hospital yard can be improved by involving users in its design. This can be done by including sufficient lighting and other design elements. The space should be enclosed but not give the impression that someone is being watched. Features should include railings and seating at various intervals, especially near the entrance, to help seniors and people with disabilities or reduced mobility.

Sound criteria

Research in four hospital gardens showed that users were disturbed by the sounds of cars, air conditioners, and traffic noise. Areas should be pre-planned to be located away from traffic, parking, and supply areas. A garden designed for therapeutic purposes should be quiet and somewhat removed from the sounds inside the hospital, which range from public announcements and television sets to catering trolleys. Visitors to the garden should feel calm and be able to hear soothing sounds, such as birds singing, wind chimes, or water flowing.

The flexibility criterion

Outdoor spaces should attract people, invite them and involve them in different activities. They need to be designed around the human factor of the users and the way these spaces are used: for a lunch break, exercise, or socializing. To maintain interest and interaction throughout the park

RESULTS AND DISCUSSIONS

The proposed solution for landscaping the premises of the Emergency Clinical Hospital "Prof. Dr. Nicolae Oblu" in Iasi aims to improve the green areas to maximize the therapeutic effects these areas can have on all beneficiaries in this health institution.

The first step in elaborating the proposal consisted of the judicious establishment of all the areas, each intended for specific categories of users. The zoning of space was done according to the needs of patients, staff, and visitors, each of these spaces having several functions. The location and connection between these areas were made according to the type of activity that can be carried out in the proposed perimeter so that the dimensions of the spaces comply with the recommended rules. Their layout aims to ensure an optimal space for each beneficiary and satisfy a potential need for privacy. The entire layout (Fig. 1) has been designed in an eclectic style. This style combines the geometric shapes of functional areas and pathways with the arrangement of green spaces and plant compositions in irregular, curved, circular, or semi-circular shapes. The resulting forms have a cumulative effect of balancing and energizing the whole space. The whole design aims to provide a pleasant ambiance and a healing environment. The design style aims to satisfy the landscape principle of unity in diversity.

In order to meet this requirement, the same types of materials were used for the furnishings, and the same types of paving materials were used for the paths and decorative pools (Dascălu and Cojocariu, 2016).

Following an overall analysis of the needs of the hospital's beneficiaries, the following functional areas were proposed in the spaces of the main building:

1. Area for road access and ecological parking;
2. Recreation area for medical and administrative staff;
3. Visitors' area;
4. Recreation and social area for patients;
5. Relaxation and meditation area;
6. Recreation and occupational therapy area;
7. Area for physical therapy and medical gymnastics;
8. Children's play area.

Zone 1 - Employee parking area

The proposed redevelopment of the car park involves the introduction of an environmentally friendly grid paving of 60 x 40 x 4 cm slab size for all parking spaces. The advantages of this type of paving are aesthetic by increasing the surface area of the pavement. However, unlike poured concrete, they are also linked to their resistance to repeated freeze and thaw cycles. The dimensions of the parking spaces are 2.5 x 5 m, arranged at 45 angles. Near the parking area, landscaping is proposed, including a decorative pond with water and plant compositions (Figure 1).

Figure 1. Functional zonation of the developed site

Zone 2 - Recreation area for medical and administrative staff

This area is primarily for medical and administrative staff (Figure 2). These people spend a large part of their day under stressful conditions, which, over time, can affect the quality of their work and their quality of life. For this reason, it is essential to have a space for relaxation and recreation in a peaceful environment surrounded by vegetation. The area is located on the SE side of the hospital building, near an entrance to the building. Access to this area is via a 2 m wide main pathway. The path of the central alley runs through all the functional areas, giving them unity.

Figure 2. Area 2 for recreation of employed staff

The area's focal point is represented by a circular pool of water enclosed at the level of the paved surface by inserts of green strips of grass. A series of wooden seating areas are arranged around this basin. From this central circular area converge two 2 m wide secondary alleys. The width of the alleys allows for the placement of new wooden seating areas for the beneficiaries of this space. The green spaces shaped by the alleys feature two plant compositions, which can be admired from the area of the wooden seats. The species of trees, shrubs, decorative grasses, and flower species used for the two compositions are: *Juniperus scopulorum*, *Acer platanoides* `Globosum`, *Thuja orientalis* `Aurea Nana`, *Acer palmatum* `Atropurpureum`, *Buxus sempervirens* `Elegantissima`, *Pinus mugo* `Pumilio`, *Juniperus horizontalis*, *Lavandula angustifolia*, *Festuca glauca*.

Zone 3 - Area for patients and their visitors

In this area, in-patients and their visitors can socialize in a pleasant environment where they can recreate. The area runs along the main pathway and in the area to the south of it. Access to the southern area is directly from the central alley, on a 1.5 m wide secondary alley. There are two significant points of interest along the secondary path. These consist of 4 semicircular basins, with seats and planters in the center (Figure 3). Also, in this area, along the route of the main path, there are a series of alcoves with wooden seats and an expansion inside which there are semicircular benches.

Figure 3. Area 3 for the recreation of patients and their visitors

The whole space is set with groups and plant compositions along the alleys, grassy spaces, and central areas of interest. In the center of the areas with semicircular basins, there are two relict compositions of the following species: *Acer palmatum* `Atropurpureum` and *Lonicera pileata*. In the garden beds bordering the water basins are specimens of *Betula pendula* `Youngii` and *Carex morrowii* `Variegata`.
In the center of the dilations with state seats, there is a composition of *Acer platanoides*

`Globosum`, *Pinus mugo* `Golden Glow`, *Pinus mugo* `Pumilio`, *Spiraea japonica* `Little Princess`, *Juniperus horizontalis* `Blue Chip`, *Phlox drummondii*, *Sedum spectabile*, *Salvia nemorosa* `Caradonna`.

Area 4 - Recreation and social area for patients
In this area, patients can spend time in nature, relax and socialize with each other. The area runs along the main pathway, with an expansion with semicircular benches and an area to the south (Figure 4). Access to the southern area is directly from the central alley, along a 1.5 m wide secondary alley.

Figure 4. Area 4 for patient recreation

In the area south of the central alley are three circular points of interest. These areas are landscaped with semi-circular pergolas with wooden benches, tables, and seating (Figure 5).

Figure 5. Detail of the center of interest in zone 4 for patient recreation

The pergola posts are decorated with *Clematis* sp. `Jackmanii` specimens, and in the center of each circular area, there are spaces for plant compositions consisting of the following species: *Hydrangea macrophylla*, *Sedum spectabile*, *Astilbe japonica*, *Salvia nemorosa* 'Caradonna', *Yucca filamentosa*, *Heuchera micrantha* 'Palace Purple', 53 *Pennisetum alopecuroides* 'Red Head'. Six clumps of *Lupinus polyphyllus* are placed along the paths.

Zone 5 - Area for relaxation and meditation
This area allowed patients to retreat to a more intimate and introverted place conducive to relaxation, meditation, and inner reflection. Access to the space is via a 1.5 m wide secondary pathway. This place has two semicircular wooden pergolas, at the base of which is a bench. In the center is a circular water basin with aquatic plants, enclosed at the level of the paved surface by inserts with green grass strips. The pergola posts are decorated with specimens of *Wisteria sinensis* to give patients color, shade and privacy (Figure 6).

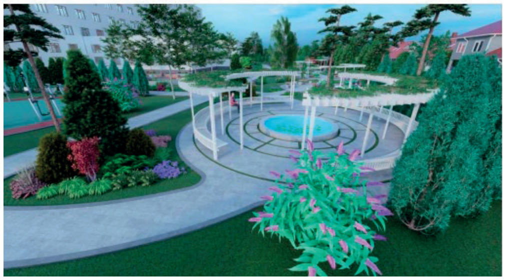

Figure 6. Zone 5 for relaxation and meditation

Two identical plant compositions, consisting of the following species, were designed in the vicinity to the north of this area: *Juniperus scopulorum*, *Thuja orientalis* `Aurea Nana`, *Pinus mugo* `Pumilio`, *Juniperus sabina*, *Berberis thunbergii* `Red Rocket`, *Heuchera micrantha* `Palace Purple`, *Euonymus fortunei* `Emerald Gaiety`, *Picea pungens* `Glauca Globosa`, *Salvia nemorosa* `Caradonna`, *Yucca filamentosa*, *Phlox drummondii*, *Pennisetum alopecuroides* `Red Head`, *Carex morrowii* `Variegata`. To screen and increase privacy in the southern part of the circular area, it is planned to introduce *Juniperus scopulorum* `Skyrocket` and *Buddleja davidii*.

Area 6 - Recreation and horticultural therapy area
This area is intended for so-called horticultural therapy, a type of occupational therapy. For this activity, a circular space has been provided. Inside are six flower boxes where various species of horticultural plants can be planted and

four wooden benches for relaxation when this space is used for recreation.

Figure 7. Area 6 for recreation and horticultural therapy

In this case, the following species were proposed for the herb and medicinal plant garden: *Ocimum basilicum* `Rubrum`, *Ocimum basilicum*, *Mentha piperita*, *Salvia officinalis*, *Rosmarinus officinalis*, *Echinacea purpurea*, *Lavandula angustifolia*. The perimeter of this circular area was planted with groups of *Hosta plantaginea* and *Hemerocallis fulva* (Figure 7).

Zone 7 - Area for physical therapy and medical gymnastics

This area is designed as a space for exercise therapy and medical gymnastics with the help of nurses and outdoor exercise equipment. These exercises allow patients to maintain or improve their physical abilities, thus speeding up healing. This space provided wooden seating areas of semicircular and circular shapes around a planter. These places facilitate socializing during the breaks patients can take between exercise sets. In order to avoid injuries in this area, it was proposed to install a flexible tartan-type slab in two different colors (Figure 8). A specimen of *Betula pendula* 'Youngii' was placed, bordered at the base by specimens of *Euonymus fortunei* 'Emerald Gold'.

Figure 8. Area 7 for physical therapy and medical gymnastics

Zone 8 - Children's play area

This area has been designed as a space for children and their visitors. In this area, child patients can play, thus being positively distracted from the many ailments and stressors they experience. The area has various swings on a surface covered with flexible tartan tiles to avoid possible injuries. Similar to the medical gymnastics area, this area also has wooden seating areas in semicircular and circular shapes around a planter, facilitating socializing and resting (Figure 9).

Figure 9. Children's play area

The green space provided to separate zones seven and eight includes, in order to partially and aesthetically screen the two zones, groups of three *Hydrangea macrophylla* specimens, provided in the basal zone with *Lonicera pileata* specimens, and a group composed of a *Cotinus coggygria* 'Royal Purple' specimen based on *Cornus alba* 'Elegantissima' specimens.

CONCLUSIONS

This project was conceived to revitalize the green spaces in the Emergency Clinical Hospital "Prof. Dr. Nicolae Oblu" premises in Iasi. Currently, these spaces are in a state of degradation, thus requiring a series of transformations to offer patients and staff the opportunity to spend quality time in a natural environment with actual healing properties. The existence of therapeutic gardens has been recorded since medieval times, and they were considered essential for relieving patients' ailments. In recent centuries, the importance of these gardens has been neglected, and concrete surfaces or car parks have replaced them. Fortunately, this holistic concept, which combines natural elements with patients' physical, psychological, and social recovery, has recently been brought back to the fore. Preliminary research clarified and systematized the main psycho-emotional and physical benefits a therapeutic garden can produce for patients and medical staff. Also, the primary criteria for designing a therapeutic garden were identified at this initial stage. A series of areas of interest to the potential beneficiary of this space was crystallized through the analysis of the study materials and the application of landscape design principles. The functional zoning of the available space on the site involved the establishment of eight zones: an area for road access and ecological parking, a recreation area for medical and administrative staff, a visitor area, a recreation and socialization area for patients, a relaxation and meditation area, a recreation and occupational therapy area, a physical therapy and medical gymnastics area and a children's play area. The design proposal considered patients' physical and psychological needs and medical and administrative staff. All these categories of beneficiaries can experience different levels of stress. This stress may be related to medical conditions in the case of patients or may be generated by the work environment in the case of staff. The design aims to provide an escape into a natural environment with healing properties and to accelerate the process of healing and emotional and spiritual balancing through spaces for rest, movement, contemplation, and meditation.

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GREEN REHABILITATION CONCEPT FOR A POST SOCIALIS MARKETPLACE (PROMOTING THE LOCAL FARMERS AND SUPPORTING THE GREEN INFRASTRUCTURE OF THE CITY OF TÂRGU MUREȘ)

Endre KENTELKY, Gabriella KISS, Ildikó LIHĂT, Róbert Marius TĂSLĂVAN, Martin JAKAB, Zsolt SZEKELY-VARGA

Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Department of Horticulture, Calea Sighișoarei 2, 540485 Târgu Mureș, Romania

Corresponding authors emails: ildiko.lihat@gmail.com, zsolt.szekely-varga@ms.sapientia.ro

Abstract

Marketplaces, are integral parts of cities all over the world. In the last decades the rapid urbanization is putting pressure on the cities, this creates numerous problems for locals. Besides over-urbanization syndrome, Târgu Mureș is still struggling with the influence of the post-socialist era, the nature of the communist vision can still be noticed. The present paper tackles the importance of marketplaces from two points of view: the sociological point of view, why it is important for locals and society; and from the point of view of ecology. The rehabilitation and re-naturalization of the marketplaces creates a pleasant meeting point for locals, helps boosting the sale of local products, strengthens the economy and supports the sustainable character of the settlement. The proposed plan can serve as a model for the renovation and development of marketplaces in dense urban areas. The researched marketplace is located close to the city center, to its historical places, and could become a good green space not only for the acquisition of goods, but for recreation space as well.

***Key words**: buy local, green infrastructure, marketplace rehabilitation, sustainable city.*

INTRODUCTION

Marketplaces play a crucial role in cities all over the world, serving not only as spaces for product sales but also as important socialization venues for local residents. However, rapid and over-urbanization in recent decades has led to increased density and reduced livability in cities. To address this, increasing green space and nature-based solutions can enhance the quality of life (Hunter et al., 2017). According to several previous studies, this can include, public parks, forests, playgrounds, sport fields, private gardens, and other green spaces (Csomós et al., 2020; Sikorska et al., 2020; Viviera et al., 2018).

Harmful human activities have damaged the environment and these effects are mostly irreversible, but they are also now part of our daily lives. Reducing their impact is a major challenge of our time. The industrial revolution led to a wave of urban development, where natural areas were transformed and "cleaned", swamps drained, rivers regulated, and riverbeds coated in concrete. All this resulted in the removal of nature from cities (Cvejić et al., 2015).

The UN World Commission on Environment and Development defined sustainable development as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). Douglas Farr's (2007) "sustainable urbanism" approach argues that urban density and connection with nature are key components of sustainable urban planning. In agreement among ecologists, economists, social scientists and planners, urban green areas can be considered as public and private spaces within cities primarily covered with vegetation, available for direct (e.g., active or passive recreation) or indirect (e.g., environmental benefits) use by the public (Koohsari et al., 2015; Kardan et al., 2015).

Târgu Mureș still struggles with the remnants of the post-socialist era, as seen even in its marketplaces (Lihăt & Ványolós, 2021; Stanilov, 2007).

Historically, the market was an integral and well-functioning part of the settlement of Târgu

Mureș and numerous fairs were held here. As even the name confirms, “târg” means “market” (in German Neumarkt, in Hungarian Marosvásárhely) (Pál-Antal, 2009).

This research examines the importance of markets from both sociological and ecological perspective. Greening market places can expand the city's urban green infrastructure and create a pleasant meeting place for locals, boost local product sales, strengthen the economy, and enhance the settlement's sustainability. The increase of green space and nature-based solutions can offer the possibility to improve the quality of life (Hunter et al., 2017).

The current study examines the history and current state of Târgu Mureș's fairs and markets and aims to transform them into community spaces that preserve traditions, re-enchant the atmosphere, and meet the needs of modern society. The proposed plan can serve as a model for renovating markets in dense urban areas. The focus of the research and study is a market located near the city center and its historical sites, which can serve as a good green space for both commerce and recreation (Figure 1).

Figure 1. The location of the analyzed market (according to: Târgu Mureș General Urban Plan 2010)

URBAN DEVELOPMENT

The first written reference to Târgu Mureș appears in a 1323 document as "Novum Forum Siculorum," meaning New Szekler Market Hall. It was officially named Târgu Mureș in 1616 and has always included "market" in its name, highlighting the significance of its fairs (Keresztes, 1996; Man, 2006).

The city developed rapidly during the 14th century and became the first Transylvanian settlement to earn the title of free royal city (Pál-Antal, 2009). Târgu Mureș was known as the fair place of Transylvania for many years and received the right to hold three national fairs from King Matthias in 1482 (Sebestyén, 2009).

The city is located in the heart of historical Transylvania, where three geographical units meet. It is a hub for exchanging grain from the Transylvanian plains, vegetables from the Niraj valley, and wooden products from the mountains (Keresztes, 1996). Weekly markets take place on Thursdays, fulfilling the needs of the city's residents and facilitating goods exchange in rural areas. Over time, fairs were added on other dates, and the city was granted the right to host three national fairs: the second Thursday after Pentecost (Lord's Day), November 11 (Saint Martin's Day), and Palm Sunday. The fairs took place in the city center, now known as the main square. As the settlement grew larger, the exchange of goods increased and the market spread to nearby areas, becoming specialized. In the early 19th century, the cattle market, wood and coal market were separated from the food market. In 1937, regulations were established for holding national fairs seven times a year (January, March, May, July, September, November, December) and weekly markets on Monday and Thursday (Sebestyén, 2011).

However, from the mid-1900s, the fairs lost importance and were moved from central squares. Several residential areas were built and smaller permanent markets were created in each neighborhood. In 1953, the main square was reorganized and the market was relocated from the city center. The goal was to have at least one market in each city quarter. The main market moved from the main square to an empty square behind the town hall, and in 1961 the daily market moved to the area between Cuza Vodă and Martinovits Street, now Belșugului Street. The market was fully developed by 1962 and modernized in 1966. (Sebestyén, 2014)

METHODOLOGY

The present research aims to find the best solution for stabilizing and expanding urban green infrastructure under a sustainable urban planning approach. This involves increasing green areas in dense urban fabric, sustaining biodiversity to reduce pollution and bring ecological, economic, and social benefits to the city and its residents. The pilot project is based on a multi-criteria analysis of the current situation of the existing marketplaces. The research aims to identify areas and methods for expanding green space, creating sustainable green spaces. The study also focuses on the relationship between the city and its inhabitants, their well-being.

ANALYSES

Romanian Regulations for Markets

The Romanian regulations for markets are outlined in the Government Decision No. 384 from 2004. This decision governs the trade of products and services in public areas such as markets, fairs, and public roads. The decision sets general rules and minimum conditions for these activities and defines trade in public areas as the sale of products and services in markets, fairs, public passages, streets, and other public areas. Article 7 of the Decision lists the types of markets that can be held in public areas, including agricultural and food markets, fairs, jigsaw fairs, mixed markets, moving markets, and flea markets.

The Decision No. 384 of 2004 regulates the operation of markets and fairs in Romania, but it does not mention the importance of market places from a sociological or environmental perspective. Although there are references to the safety of the population and rest areas, there is no discussion from a sustainable cities approach, that is so important nowadays (Romanian Government, 2004).

Settlement-level analyses

During the analysis of Târgu Mureș markets, their locations were marked. The agro-food markets are located in central parts of larger residential areas, except for the Cuza Vodă street market, which is situated near the historical city center, not in a specific residential area.

The markets' accessibility was evaluated through the bus network analysis, showing that each market has at least one bus stop within a maximum distance of 400 m and, in some cases, multiple bus stops, making it easy to access the markets by a short walk. A population analysis was also conducted, indicating that each market is located in an area with high population density of over 1,000 inhabitants/km^2 (Târgu Mureș City Hall, 2010)

General analyses of examined markets

During the visits to the city's markets, have been recorded observations of their size, type of roofing, use of greenery, availability of parking places, and accessibility by public transportation. The results are summarized in Table 1 below (Târgu Mureș Administration of markets, 2021).

Table 1. General dates from markets (Târgu Mureș Administration of markets, 2021)

Market name	Area (m^2)	Covered zone (m^2)	Material of the pavement	Parking lot	Nearest bus stop (m)
Cuza Vodă	12429	1100	asphalt, pavement	yes	~ 30
Unirii	790	220	asphalt, pavement	yes	~ 120
1848	4234	2000	asphalt	yes	~ 400
1989 dec. 22	9399	2600	asphalt, pavement	yes	~ 320
Diamant	1124	1087	asphalt	no	~ 240
Dacia	1148	600	asphalt	no	~ 230
Armatei	7461	2640	asphalt, pavement	no	~ 480
Vechituri Market	23000	-	asphalt, pavement, gravel cover	no	~ 440

Typology of the analyzed marketplaces

According to Article 7 of Decision No. 384 from 2004 two types of markets can be distinguished in Târgu Mureș: permanent markets and periodic fairs. Permanent markets are those that operate on a daily or weekly basis, and are established in a specific location for a longer period of time. Periodic fairs, on the other hand, are temporary events that occur periodically, usually for a limited timespan and in a different location each time (Romanian Government, 2004).

1. Permanent markets

There are currently eight permanent markets in the city, six of which sell agro-food and industrial products. These include: the daily market on Cuza Vodă Street, the Unirii quarter market, the “1848 market” in Dâmbu Pietros residential area, the “December 22 1989” market in the 7 Noiembrie district, the “Diamant” and “Dacia” markets in the Tudor Vladimirescu quarter and the market of Mureșeni residential area. In addition to these, the city also has two non-food industrial markets: the Armatei market ("Russian market") from Armatei square and the "Piața de Vechituri" market, which functions as a flea market and is open only on Sundays (Figure 2).

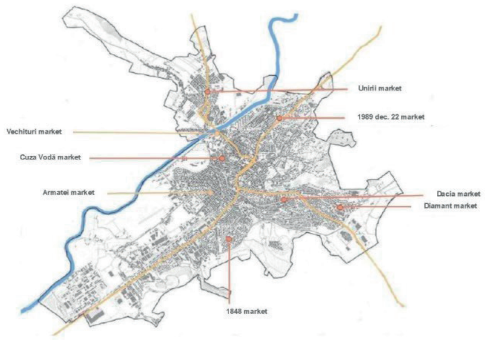

Figure 2. Location of the permanent markets (according to: Târgu Mureș General Urban Plan, 2010)

The markets in Târgu-Mureș trade in various products and services including vegetables and fruits, household goods, food industry products, non-food industry products, flowers, smaller animals (such as lambs), and catering. The market is divided into sectors for agricultural producers, household goods, food industry products, non-food industry products, flowers, live animals (with a mini-slaughterhouse for lambs), and catering (Târgu Mureș Administration of markets, 2021).

2. Periodic fairs

In addition to permanent markets, Târgu Mureș also holds periodic outdoor fairs several times a year, usually during holidays. These fairs can be one-day events or last several days or even a week. Examples include the Christmas fair, the Women's Day flower fair, the newly organized Easter fair, the "Local Farmers Market" organized by Petry, and the craft fair called "Târgul Cetății." These fairs are usually located in the central part of the city and are highly popular, attracting many visitors and adding vibrancy to the city's daily life. (Târgu Mureș Administration of markets, 2021).

CASE STUDY

Landscape analyses

1. Existing situation

The Cuza Vodă Street market in Târgu Mureș is located in the central part of the city and surrounded by Cuza Vodă and Belșugului streets. It opened during the summer of 1961; before operating on Cuza Vodă Street, the market was located in a non-equipped square behind the City Hall, having been moved from the main square (then Stalin square). The present area was completed in 1962 and modernized in 1966 with covered counters. In 1976, a covered market hall was built.

The market on Cuza Vodă Street in Târgu Mureș is the largest of the city's six agri-food markets, covering 12,429 m^2. It is not a residential market but the central, "big market" visited by people from all over the city. The market operates daily and is open seven days a week. It has high turnover, with fewer visitors on Mondays and the most from Thursday to Saturday. According to Google data's, the average visit is 20 minutes per costumer.

2. Accessibility

The market has two entrances, one on Cuza Vodă Street (pedestrian only), this is the main, and one on Belsugului Street (with a 50-car parking lot). Accessible via public transport with a nearby bus stop on Cuza Vodă Street (30 meters from main entrance). Surrounded by a pharmaceutical factory, kindergarten, small grocery store, bakery, and residential buildings.

3. Function analyses

The market area is dominated by its market function, with both outdoor and indoor sales. Additionally, there are buildings with administrative functions, several shops, a small rest area, and a mini-slaughterhouse that operates periodically, specifically during Easter.

4. Built-in, paved, green spaces

The market area is largely paved, images can be seen in Figure 3, to accommodate its market function and high pedestrian traffic, but this has negative impacts on the microclimate due to the limited green space and very few plants.

Figure 3. Existing pavement

The built-up areas occupy 34% of the total area, while paved surfaces make up 65%, split almost equally between asphalt and concrete sponge. The proportion of green space is low, only 0.21% of the total area. This exacerbates the heat-island effect, a common phenomenon during hot summer days (Figure 4).

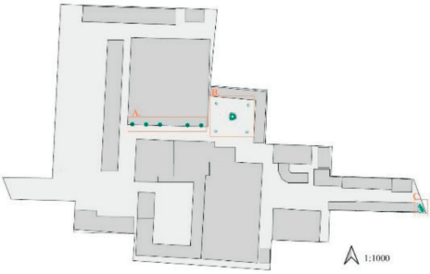

Figure 4. Existing green areas

5. *Outdoor furniture*

There is a diverse range of outdoor furniture available, some of which are specified in market regulations, such as vegetable washers and blue-painted metal market sales counters. The two drinking fountains in the area are in poor, neglected condition. The resting area includes a few wooden benches and plastic trash cans, but these furnishings are not cohesive in terms of color, material, or design (Figure 5).

Figure 5. Existing furniture

6. *Existing vegetation*

The market's vegetation is limited, with a small number of plants on its few green areas. On the paved surfaces, there are a few trees in pots and four common ash trees (*Fraxinus excelsior*) at the corners of the resting area, as well as five arborvitaes (*Thuja occidentalis*) on one side of the outdoor covered sales area.

The resting area has a small circular garden with a rose bush (*Rosa* sp.) in the center surrounded by creeping junipers (*Juniperus horizontalis*), three common boxwoods (*Buxus sempervirens*), and wax begonias (*Begonia semperflorens*). At the entrance from Cuza Vodă street, there is another small area with Japanese spindles (*Euonymus japonicus*), creeping junipers (*Juniperus horizontalis*). The green areas are limited in size and have limited vegetation, with most of the plants not being native to the area (Figure 6).

Figure 6. Existing vegetation

7. *SWOT analyses*

To summarize the analysis, a SWOT analysis was prepared for the planning area. The SWOT analysis shows there are many strengths and opportunities in the area that can support sustainable city development, but also many weaknesses that could create additional problems and threats if not addressed (Figure 7).

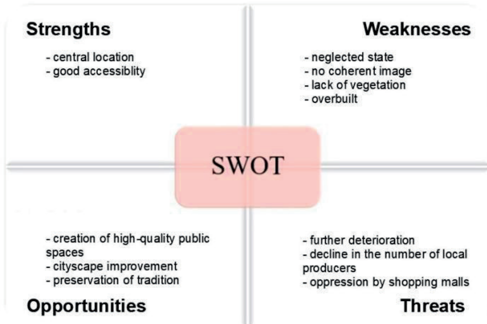

Figure 7. The SWOT analysis

Presentation of the concept

The development of the concept was facilitated through multiple visits on the site, conducting visual tests and analyses, and reviewing relevant case studies. A glaring deficiency observed in the area was the insufficiency of vegetation, which impairs the space both aesthetically and ecologically. The concept aims to address this issue through a primary focus on greening. However, augmenting the green area presents a considerable challenge as the area is situated in a highly developed central district and features a large paved surface. This led to the proposal of utilizing green roofs and green walls to bring nature back into the urban environment and its associated benefits like supporting the biodiversity, better rain water management, ecological benefits, etc. (Pál., 2008).

In line with the greening theme, the design prioritizes sustainability and environmental responsibility, recognizing the critical role it plays in contemporary open space design. This includes considerations such as the use of eco-friendly materials, transportation, and the management of water and energy.

The concept is visually represented in the accompanying flowchart (Figure 8).

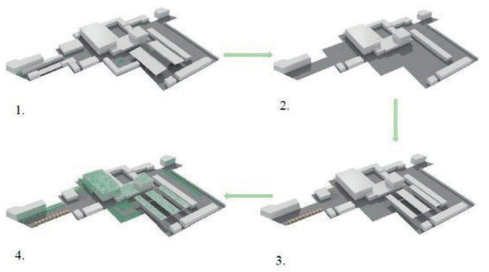

Figure 8. Concept flowchart

The initial illustration represents the existing conditions, characterized by the scarcity of green spaces and a high concentration of built environments. The subsequent figure depicts the scenario after the demolition or reconstruction of specified structures. These buildings were recommended for removal as they obstruct views, serve non-essential functions, and contribute to the prevalence of built-up and paved spaces, leading to a feeling of congestion. In the third illustration, the newly proposed constructions have been introduced. Finally, the fourth figure, depicts the area after the introduction of vegetation, creating a more livable green space for the city.

Extending this concept to the city-level, by applying similar transformations to agricultural-food markets (Figure 9), the city could potentially boast six such livable green spaces located in under-served areas, providing a positive impact on the urban landscape, helping the sustainable urbanism theory (Haq, 2011).

Figure 9. City-level concept (according to: Târgu Mureș General Urban Plan, 2010)

The plan proposes incorporating green roofs and walls in the densely built and fully paved urban market square. The intensive green roof design, recommended for the roof structure above the sales counters, is suggested to replace the current polycarbonate roof due to its poor performance in providing shade and its overheating in summer. It is recommended to utilize a reinforced concrete structure with sufficient load capacity for the establishment of an intensive roof garden covering an area of 1,620 m^2. To provide light and shade, four 6 x 6 m skylight windows will be installed. Access to the roof garden will be facilitated through a staircase, two additional ladders will be placed for emergency purposes. The roof garden will feature perennial plantings, trees, shrubs, lawns, benches, drinking fountains, and other elements to create a relaxing and green environment for visitors (Figure 10).

Figure 10. Aerial perspective of the proposed design

In the proposed design, the indoor market building is equipped with a semi-intensive green roof, given the unknown load-bearing capacity of the existing building. The lighter structure of a semi-intensive green roof allows for the provision of a garden-like experience for visitors. The south-eastern part of the roof

features a paved space, complete with benches and tables, affording views of the main square and its notable landmarks, such as the town hall tower. Safety barriers have been incorporated at accessible areas of the roof. Access to the semi-intensive green roof is possible via a staircase from the indoor market building, as well as through the staircase shared with the intensive green roof. The adjacent building block, which is of a lower elevation, features an extensive green roof, with limited accessibility but important ecological and aesthetic functions. The northernmost portion of the building block features an extensive green roof and the placement of solar panels.
In addition to the implementation of green roofs, the utilization of green walls is also proposed in the plan. The concrete fence along the northern border of the parking lot and the fire wall adjacent to the entrance from Belșugului Street would be covered with vegetation. The upper part of the indoor market building would also receive green wall treatments. A modular green wall with a diverse range of plant species is recommended for the fire wall at the entrance from Cuza Vodă Street, serving as a prominent and visually appealing feature at the square's major point of entry.
Furthermore, the area surrounding the market is proposed to receive tree planting, including the entrance area, where benches, drinking fountains, and a row of narrow-crowned trees would replace the existing shop premises. Additionally, a small space in front of the outdoor market is envisioned to include tree planting, creating a rest area, and the possibility of hosting stalls selling products periodically. A row of trees in the middle of the parking lot is also proposed to provide shade to the area. A parallel bicycle path and bike parking lot would be established at the back entrance. Finally, the upgrade of pavements and street furniture is also recommended. All this are shown in Figure 11.

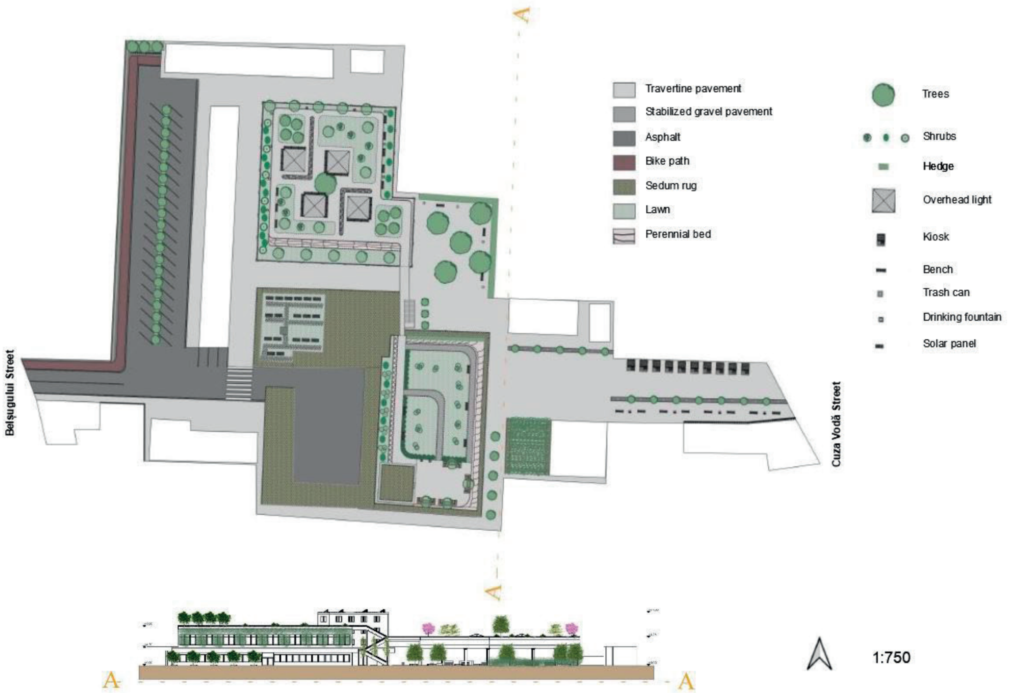

Figure 11. Masterplan and section of the proposed design

1. Proposed pavements
For the pedestrian areas of the market, with the exception of the vehicle access points such as the rear entrance, parking lot, and loading area, the utilization of travertine stone slab surface is proposed. This material is naturally occurring, if it is accordingly treated is resistant to freeze-thaw cycles, and is durable, while its light-

colored appearance adds aesthetic appeal to the area. From the main entrance to the cafe, and extending to the right, the incorporating granite cube stone paving is suggested, in one-meter sections. This design element is important from the point of view of rainwater management intended to facilitate percolation of precipitation to the roots of the planned rows of trees and add a touch of variety to the otherwise uniform travertine cladding.

The surfaces of both the intensive green roof and the sidewalk connecting ramps and paved areas on the semi-intensive green roof would also be covered with travertine tiles for aesthetic consistency. Meanwhile, the less trafficked areas of the semi-intensive green roof would feature a stabilized gravel pavement for its natural appearance.

As for the rear entrance and parking lot, the existing asphalt pavement would be retained due to its good condition, a more resistant pavement is needed for heavy traffic. The rainwater would be directed to the tree row roots area from this surface. Adjacent to the parking lot, a bike path featuring a molded rubber surface is proposed.

2. Proposed vegetation

From the entrance on Cuza Vodă Street, a row of arboreal specimens on the left side of the site is devised, continuing on the right side; it provides an aesthetic experience for those who are passing by outside (Schmidt & Fekete, 2003). In this area, a tree species with a narrow crown is chosen, as the location serves as a thoroughfare and it is desirable to minimize encroachment without sacrificing shading and visual interest. To this end, common aspen (*Populus tremula* 'Erecta') is selected. In proximity to the indoor market building, is incorporated spherical flowering ash (*Fraxinus ornus* 'Mecsek'), as this species offers attractive ornamental qualities and is tolerant to urban environments. For the remaining area, planting the goldenrain tree (*Koelreutreia paniculata*) it is recommended, which is a distinctive ornamental species due to its fruit. For the parking area, a tree species that would not grow to a substantial height is proposed due to overhead power lines, but would still afford a visually appealing row of trees, thus the globular Norway maple (*Acer platanoides* 'Globosum') is chosen grafted onto a tall trunk. English ivy (*Hedera helix*) and Virginia creeper (*Parthenocissus quinquefolia*) would be utilized for the trailing vegetation walls (Figure 12).

Figure 12. Render of the proposed green roofs

On the modular green wall, installing the cultivars is proposed, with the following perennials of the coral bells (*Heuchera), H.* 'Palace Purple'*, H.* 'Lime Marmalade'*, H.* 'Kassandra'*, H.* 'Blondie in Lime'*,* and *H.* 'Cherry Cola'. These species are hardy in winter and even retain their foliage during milder conditions, thus also providing ornamental value.
For the extensive green roof, a carpet of stonecrops (*Sedum*) is proposed, consisting of *Sedum album*, *S. sexangulare*, *S. acre*, *S. hybridum*, and *S. spurium*. As regards the intensive and semi-intensive green roofs, carefully selected plant species are appropriate for roof top cultivation, such as those with non-invasive root systems, low water and nutrient requirements, tolerance to direct sunlight, and frost hardiness. Furthermore, species that would provide year-round ornamental value are chosen, including *Acer ginnala*, *Koelreuteria paniculata*, *Magnolia kobus*, *Malus x purpurea* as trees, *Juniperus squamata*, *Spirea x vanhouttei*, *Weigela florida* 'Variegata' for shrubs, and a perennial layer consisting of *Aster novi-belgii*, *Aster dumosus*, *Centranthus Centranthus ruber*, *Echinacea purpurea*, *Kniphofia Knipophia uvaria*, *Perovskia atriplicifolia*, and *Saponaria officinalis*.
3. Exterior furniture
It is recommended to replace the existing outdoor furnishings in the marketplace, as they are in a deteriorated state. A new outdoor furnishings proposal is to use natural materials such as wood, metal, and stone, and incorporate them into harmonious designs. The vegetable stands, sales counters, drinking fountains, and trash receptacles, which are made of metal for longevity, will be coated with pine wood for a more natural look.
The seating surfaces of benches will be composed of wood, with stone accents. The design of sales counters will remain unchanged, but the materials will be altered to incorporate a combination of wood and metal. The form of the vegetable washers will remain the same, but their construction materials will also be altered to match the sales counters. The drinking fountain, trash receptacle, bench, and plant box will be incorporated into the design to create a unique set of elements, which will be placed in the market area as depicted in Figure 13.

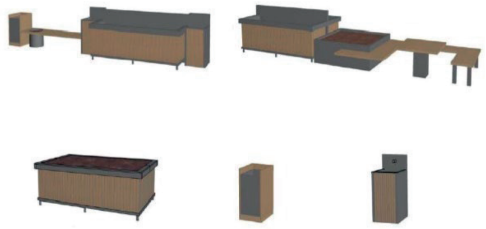

Figure 13. Proposed exterior furniture

CONCLUSIONS

The objective of the study was to examine the marketplaces of Târgu-Mureș, their historical background, and to formulate strategies to enhance the local green infrastructure in order to attain the goal of creating a more livable city through sustainable landscape architectural and urban design approaches.
The findings of the research substantiated the need for a comprehensive approach towards these spaces as they present numerous opportunities for sophisticated planning and design. The research culminated in the conclusion that marketplaces are integral components of urban open spaces, and should be considered not only as places for the acquisition of goods, but also as recreational spaces due to their unique and important function in the densely built urban landscape. The incorporation of green spaces and vegetation can serve as a recreational and aesthetic enhancement to the marketplaces.
The study also identified a lack of green spaces and vegetation as a significant challenge, and presented sustainable urban planning solutions to address this issue. This included the demonstration of various alternatives for increasing green space through description, presentation, and detailing. The addition of green elements to the grey surfaces of urban areas has been shown to significantly improve the microclimate quality, particularly in market areas where the heat island effect can be a concern. Green surfaces help to raise humidity levels through evapotranspiration, while also promoting air cleanliness by binding carbon dioxide and air particles. Such findings highlight the importance of incorporating green infrastructure into urban planning and design to enhance both environmental and human health

outcomes. The objective of the pilot plan is to develop a marketplace that functions as both a shopping destination and a social gathering place while incorporating principles of sustainability and green design to promote its viability as an urban area. This aligns with the perspective of Jan Gehl, as expressed in his book "Cities for People," which posits that a livable city is characterized by its thriving community, ecological health, and aesthetic appeal, all of which contribute to the psychological and aesthetic well-being of its residents (Gehl, 2014).

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RESPONSE OF ZINNIA PLANTS TO FOLIAR APPLICATION OF SALICYLIC ACID

Carmen NICU, Manuela MANDA

University of Craiova, Faculty of Horticulture, 13 Al. I. Cuza Street, Craiova, Romania

Corresponding author email: manda_manu@yahoo.com

Abstract

Zinnia elegans Jacq. is one of the most popular annual flowering plants, highly appreciated due to the great diversity of flower sizes, shapes and colours, as well as abundant blooming throughout the summer. It is widely used in gardens, and as a cut flower in bouquets and floral arrangements, but can also be grown in containers and pots, for patios and balconies decoration. This study was conducted to evaluate the effect of exogenous salicylic acid (SA) applied in different concentrations (0, 75, 100 and 200 ppm), on the vegetative growth and flowering of this plant. The results showed that foliar application of salicylic acid had a positive effect on the main analysed morphological characteristics. The maximum values of plant height, number of branches per plant, leaf length and width, number of flowers and flower diameter were obtained in plants sprayed with 200 ppm SA, compared to control plants. The salicylic acid induced early flowering, the lowest number of days required to opening of the first flower was recorded at 100 ppm concentration.

***Key words**: Zinnia, salicylic acid, foliar application.*

INTRODUCTION

Zinnia elegans Jacq. (Compositae family) is an important annual garden plant, native to Mexico, with upright, vigorous, branching and hairy stem, that can reach 15-100 cm in height, depending on the type and cultivar. The leaves are simple, entire, opposite, sessile, ovate to lanceolate, up to 10-12 cm long, covered with short hairs and rough to the touch. There are numerous beautiful varieties, with a wide range of shapes, sizes and colours of flowers. The solitary inflorescences form at the end of each branch and can be single, semi-double or double, in different shades of white, pink, red, yellow, orange, green, purple, even bicoloured or with interesting stripes and speckles. The attractive small inflorescences of the dwarf cultivars, that have 3-6 cm in diameter, or the spectacular extra-large inflorescences of 10-15 cm diameter, attract the beneficial insects as bees and butterflies. Zinnias bloom continuously throughout the summer until autumn or to the first frosts. It prefers sandy, loamy, moderate moisture soils, well-drained, rich in organic matter and mineral elements. Zinnias grow best and flower abundantly in sunny places, protected from the wind, and they are heat and drought-tolerant plants, but very sensitive to low temperatures.

It very easily propagated by seeds sown outdoors, directly in the ground after the last spring frost has passed or indoors from February until April.

Zinnia is one of the most appreciated and cultivated annual plants for the long period of flowering and the abundance of flowers in various, intense and bright colours, that bring visual interest in parks, gardens and urban green spaces, for the fast growth and the low maintenance requirements. The dwarf and compact varieties are used in flower beds, borders, mass plantings, but can also be planted in window boxes, as well as in pots and containers for decorating patios, terraces and balconies. The tall varieties are very attractive in mixed borders, groups on lawns or along a walkway, and as cut flowers in bouquets and flower arrangements.

Salicylic acid (SA) is an important phytohormone, present naturally in very low concentrations in plant tissues, which has an essential role in the regulation of various physiological and biochemical processes such as membrane permeability, uptake and transport of ions, antioxidant enzymes activities, stomatal closure, inhibition of ethylene biosynthesis, thermogenesis, photosynthetic rate, increasing of chlorophyll and carotenoid pigments content, etc. (Hayat & Ahmad, 2007).

Numerous studies worldwide had as objective to determine the responses of plants to adverse environmental conditions and the role of salicylic acid in reducing or mitigating the negative effects of different biotic and abiotic stress factors, which reduce the growth and development of the plants, and also limit the crop productivity.

The application of exogenous salicylic acid has been shown to be beneficial for plants both in optimal and stress environments (Khan et al., 2015), and can provide protection of plants by improving the tolerance to various types of abiotic stresses (Koo et al., 2020), such as drought (Zarghami et al., 2014; Yao et al., 2016; Zargarian et al., 2016; Abaspour Esfaden et al., 2019; Abbas et al., 2019; Akhtar et al., 2022), low temperatures (cold and chilling stress) (Promyou et al., 2012; Huang et al., 2016), heat (Cao & Li, 2014; Shen et al., 2016), salinity (Kamali et al., 2012; Zheng et al., 2018; Abd El Gayed, 2020), heavy-metal contaminants (El-Tayeb et al., 2006; Sharma et al., 2020), ultraviolet radiation (Liu et al., 2022; Zheng et al., 2022), nutritional deficiency (Guo et al., 2019), and inducing the plant resistance to different diseases (Durner et al., 1997; Kumar, 2014). The positive effects of salicylic acid foliar application on some ornamental plants grown both under normal and different stress conditions have been reported in many studies. It was found that SA improved the germination of *Zinnia elegans* (Huang et al., 2015) and *Limonium bicolor* seeds (Liu et al., 2019), promoted the rooting of poinsettia and azalea cuttings (Sardoei et al., 2014; Hou et al., 2020), enhanced various vegetative and flowering parameters like the plant height, the number of branches per plant, the leaf area and number of leaves, the number and diameter of flowers in *Antirrhinum majus* (Akram et al., 2022), *Calendula officinalis* (Bayat et al., 2012), *Ixora coccinea* (Gad et al., 2016), *Cyclamen persicum* (Farjadi-Shakib et al., 2012), *Saintpaulia ionantha* (Jabbarzadeh et al., 2009), *Gladiolus grandiflora* (Pal et al., 2015), *Tagetes* sp. (Poudel & Subedi, 2020), *Impatiens walleriana* (Safari et al., 2022), as well as the productivity of ornamental and horticultural plants (Larqué-Saavedra & Martin-Méx, 2007; Hayat et al., 2010), and induced early flowering in *Sinningia speciosa* (Martín-Mex et al., 2015). SA also delayed flower senescence and extended the vase life of rose cut flowers (Zamani et al., 2011), carnation (Roodbaraky et al., 2012), zinnia (Iqbal et al., 2012), chrysanthemum (Mashhadian et al., 2012), lisianthus (Bahrami et al., 2013), gerbera (Mehdikhah et al., 2016), gladiolus (Saeed et al., 2016), tuberose (Ezz et al., 2018), sunflower (Othman & Esmail, 2020), alstroemeria (Langroudi et al., 2020).

The objective of this study was to evaluate the efficiency of salicylic acid foliar application on growth and flowering characteristics of the *Zinnia elegans* plants.

MATERIALS AND METHODS

The study was conducted in the Floriculture Research Area, Faculty of Horticulture, University of Craiova, Romania, to evaluate the influence of salicylic acid applied by foliar spraying, in improving the growth of plants and flower quality. The zinnia seeds were purchased from Agrosel seed company and were sown in March 2019, under greenhouse conditions, in plastic trays, filled with a permeable sowing substrate, containing sphagnum white peat (ProfiMix, Agro CS). The healthy and uniform size seedlings were transplanted at the four-leaf stage, in a growing substrate composed of a peat and perlite mixture (1:1), in 10 cm diameter plastic pots. In May, the vigorous seedlings were planted in open field, at a distance of 30 cm between rows and 20 cm between plants along the row. After 15 days from the planting in soil, foliar treatments with salicylic acid (SA) were applied in three concentrations (75, 100 and 200 ppm), while the control plants were sprayed at the same time with tap water, early in the morning, using a manual sprayer.

The foliar applications with SA were repeated two times, at an interval of one week, during the vegetative stage of plants. The experimental observations on vegetative and flowering parameters included the plant height (cm), number of branches per plant, length and width of the leaves (cm), number of flowers and flower diameter (cm), as well as the number of days from sowing to flowering.

The experiment was arranged in randomized complete block design with three replications. The statistical analysis of recorded data was performed by one-way analysis of variance (ANOVA) for each parameter studied, and the differences between the means of the treatments were compared using Duncan's multiple range test (DMRT) at the 5% probability level.

RESULTS AND DISCUSSIONS

Vegetative growth parameters

The variability of growth parameters of zinnia plants in response to salicylic acid foliar application is shown in table 1. In terms of the average plant height, the higher values than the control plants (41.66 cm) were obtained in all variants, but the highest value was recorded by the plants sprayed with 200 ppm SA (53.50 cm), where the height increased significantly in comparison with the other concentrations applied, and these results are in agreement with those reported by Zeb et al. (2017).

The efficacy of exogenous SA on plant growth and development, depends on many factors as the concentration used, the species and plant developmental stage, timing and methods of application (soaking the seeds before sowing, irrigating or foliar spraying), number of applications, the endogenous level of salicylic acid in plant, etc. (Horváth et al., 2007; Li et al., 2022). Elbohy et al. (2018) reported a significant increase of plant height, number of flowers and flower diameter in zinnia plants grown in open field and sprayed with 300 ppm SA. In another study, Al-Abbasi et al. (2015) observed similar growth promoting responses at lower concentrations of SA (50 mg L^{-1}). Basit et al. (2018) evaluated the effect of exogenous application of different doses of SA on marigold (*Tagetes* sp.) in greenhouse conditions, and concluded that for better growth and flower production, the plants should be sprayed before flowering stage with 120 mg/L SA solution.

Regarding the average number of branches per plant, the statistical analysis of the data indicated a positive significant influence of exogenous SA application, at all treatments compared to the control plants. The maximum number of branches (5.46) was noticed at 200 ppm SA application, which was found statistically similar to 100 ppm concentration (4.78), while the minimum value of this vegetative parameter was recorded in the untreated plants. The leaf sizes generally increased, but salicylic acid spraying in different concentration had no statistically significant effect on the length and width of the leaves.

In many studies, it has been suggested that the beneficial effect of SA on plant growth, could be related to its action in stimulating chlorophyll synthesis, the improvement of photosynthetic rate and stomatal conductance, as well as in the activity of some important enzymes. SA interacts with other plant hormones, such as auxins, gibberellins, cytokinines and ethylene, to regulate plant growth by modulating cell division and tissue expansion in roots and stems (Arif et al., 2020).

Table 1. The effect of foliar application of salicylic acid on the vegetative growth of *Zinnia elegans* plants

Salicylic acid concentration (ppm)	Plant height (cm)	Number of branches/ plant	Leaf length (cm)	Leaf width (cm)
0	41.66 b	2.63 d	9.75 a	4.11 a
75	42.83 b	3.81 c	10.29 a	4.53 a
100	45.16 b	4.78 ab	10.63 a	4.86 a
200	53.50 a	5.46 a	11.12 a	5.25 a

Means with same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan's multiple range test.

Flowering parameters

Data presented in table 2 show that foliar application of salicylic acid caused a significant increase in number of flowers per plant. The plants treated with salicylic acid in 200 ppm concentration had the highest number of flowers (6.41), while at the plants sprayed only with tape water was recorded the lowest number of flowers (3.25). Similar results have been reported in *Gazania rigens* L. (El-Shanhorey & Hassan, 2021), *Lilium* cv. Tresor (Pahare & Beura, 2022), *Calendula officinalis* L. (Pacheco et al., 2013). There were no significant differences between treatments concerning the diameter of flowers. The foliar spraying of salicylic acid determined significant decreases in number of days to flowering. The comparison of average values revealed that the treated plants had an earlier flowering. The minimum value for number of days from sowing to flowering was recorded at the treatment with SA in 100 ppm concentration (60.84), followed by the variant where 200 ppm SA was applied (62.51). The flowering was delayed in control plants, where no salicylic acid was applied and the highest number of days to opening of the first flower (66.45 days) was recorded.

Flowering is an important parameter that is directly related to yield and productivity of plants (Hayat et al., 2010).

The exogenous application of salicylic acid stimulates flower bud formation and induces early flowering in different ornamental plant species, because accelerates biosynthesis of secondary metabolites and acts as a regulator of flowering time in non-stressed plants (Martínez et al., 2004). Alwan et al. (2018) showed that the soaking of *Iris hollandica* bulbs in the distilled water prior to planting led to delay in the time of flowering (113.80 days), in comparison to those were soaked in 200 mg L^{-1} salicylic acid, where the flowering occurred much earlier, after only 100 days. Martín-Mex et al. (2010) found that salicylic acid at 1 µM induced early flowering with six days, and also increased the number of flowers in *Petunia hybrida.*

Table 2. The effect of salicylic acid on flowering of *Zinnia elegans* plants

Salicylic acid concentration (ppm)	Number of flowers	Flower diameter (cm)	Number of days to flowering (day)
0	3.25 c	6.61 a	66.45 d
75	4.37 bc	6.94 a	64.72 c
100	4.62 b	7.22 a	60.84 a
200	6.41 a	7.53 a	62.51 b

Means with same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan’s multiple range test.

CONCLUSIONS

The foliar application of salicylic acid in the vegetative growth stage had a positive effect on zinnia plants and induced the enhancement of the growth and flowering parameters. The results showed that spraying with 200 ppm SA significantly increased the plant height, the number of branches per plant and the number of flowers compared to the control plants, while salicylic acid applied in concentration of 100 ppm reduced the number of days to flowering and determined an earlier flowering of the plants.

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CHARACTERIZATION AND IDENTIFICATION OF GENETIC DIVERSITY AMONG ROSE GENOTYPES USING MORPHOLOGICAL AND MOLECULAR MARKERS

Diana Patricia OARGĂ (PORUMB)[1], Monica HÂRŢA[1], Mihaela CORNEA-CIPCIGAN[1], Doina Angela PUI[2], Gabriela ROMAN[3], Mirela Irina CORDEA[1]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture and Business in Rural Development, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania
[2]Al. Borza Botanical Garden Cluj-Napoca, 42 Republicii Street, 400015, Cluj, Romania
[3]Horticultural Research Station, 5 Horticultorilor Street, 400394, Cluj, Romania

Corresponding authors emails: mcordea@usamvcluj.ro, monica.harta@usamvcluj.ro

Abstract

The aim of this research was to investigate the genetic variability of Rosa species and cultivars using morphological traits and PCR-based start codon targeted (SCoT) molecular markers to determine their degree of relatedness. Morphological analysis revealed significant differences between the genotypes. Thus, the rosehip fruit diameter ranged between 16.4 mm (R. canina Baisoara) and 26.5 mm (R. rugosa UASVM Cluj-Napoca), whereas the fruits length ranged between 33.7-27.2 mm for R. canina and 19.1-20.2 mm for R. rugosa. Regarding the number of seeds/fruit, the highest average number was registered in R. canina Salina Turda (30.05) and the lowest in R. rugosa UASVM Cluj-Napoca (17.15). Analysis of SCoT bands revealed a polymorphism ranging from 62.5% (SCoT 8) to 81.81% (SCoT 2). The PCoA and PCA analysis plots showed a tight grouping of the R. canina genotypes, distinct from the others Rosa sp. The cluster analysis divided the samples into two groups: the first comprised R. galica, R. rugosa, and R. damascena, while the second consisted of R. canina. Significant variations in each subgroup may be attributed to the samples' origins, and to morphological and genetic differences. These results provide new perspectives for exploiting the existing genetic variability among the evaluated species and further using them for ornamental, pharmaceutical, and cosmetic purposes.

Key words: *morphological traits, multivariate analysis, R. canina, rosehip, SCoT analysis.*

INTRODUCTION

There are approximately 200 species in the genus and subgenus *Rosa*, which are categorized and divided into ten sections (Wissemann, 2017).

In the Romanian flora, there are a number of 29 documented spontaneous and subspontaneous species, with an additional of five *Rosa* L. genus hybrids. Out of the 29 species, 16 species have been identified in the northeastern region of Romania (Oprea 2005).

The *Rosa* genus includes a variety of species that have served humanity as an economic resource, food, and medicine throughout history (Mármol et al., 2017). The therapeutic potential of *Rosa* species include anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, antibiotic, analgesic, antidiabetic, neuroprotective, anti-hyperlipidemic, genoprotective, and anti-obesity properties (Khazaei et al., 2020). These properties are due to the fruits' significant content of bioactive compounds and nutritional properties. The composition of the fruit, and, consequently, its derived products, varies according to the species, agricultural practices, cultivation area, as well as the time of harvest (Mannozzi et al., 2020). Furthermore, multiple variables, such as genotype, variety, growth and harvest location, growth conditions, harvesting time, maturity phases, and climatic conditions (i.e., temperature changes, and ultraviolet radiation), may have an impact on the morphological parameters and genetic diversity of rosehips (Benković-Lačić et al., 2022, Medveckienė et al., 2023). This highlights the importance of understanding the genetic variability of *Rosa* species and cultivars using molecular markers, which can help optimize cultivation and harvesting practices to maximize their medicinal potential (Agarwal et al., 2019). Throughout history, different plant parts of *Rosa* genus have been used for several purposes, including for nutritional, medicinal, and economic resources. *Rosa* species have been used historically since the Hippocratic era in ancient Greece when *Rosa canina* was recommended as a treatment for injuries caused by animals. Traditional Persian medicine records suggested the use of *Rosa canina* as a cure against several ailments, including headaches, gastrointestinal, and neurological disorders (Khazaei et al., 2020).

Currently, traditional remedies enjoy a high degree of trust from public, who often believe that natural products are inherently beneficial to the body and present fewer side effects compared to synthetic medication. Conversely, health professionals are reticent regarding recommending these products, highlighting the lack of standardization of available formulas as well as the absence of rigorous scientific documentation, such as clinical trials, necessary to establish the efficiency, safety profile, and dosage of administration (Ernst, 2000; Fuhrmann et al., 2010; Landis et al., 2014).

Species of the *Rosa* sect. Caninae are primarily odd polyploids, most frequently pentaploids (2n = 5x = 35), but their reproduction is sexual. This necessitates meiotic division, which is conventionally complicated by odd polyploid genetic material. To overcome this obstacle, species with an asymmetric polyploid chromosomal formula resort to apomixis: a phenomenon of asexual seed production at the maternal tissue level. *Rosa* sect. caninae represents a rare exception to this rule, benefiting from a unique meiotic process known in the specialized literature as "caninae meiosis" (Kovarik et al., 2008).

Prior research has also demonstrated a considerable degree of individual variation within the *Rosa* species. Future investigations on the development of rosehip cultivars may take into account the potential existing ecotypes. Wild edible fruits have distinct gene combinations in addition to a higher degree of gene diversity (Okatan et al., 2019). It is generally known that agricultural genetic diversity is increased by indigenous species (Bozhuyuk et al., 2021). The genetic diversity of wild fruits is projected to expand as a result of frequent propagation of seeds and thorough out-crossing. A study exploring *Rosa canina* L. fruits from the Oltenia region revealed a significant degree of variability in morphological characteristics and biochemical composition of fruits from spontaneous flora (Soare et al., 2015).

Due to pronounced polymorphism and interspecific hybridization, the morphological classification of species within the *Rosa* genus has not resulted in globally accepted systematization. This assessment has been supplemented by information obtained through anatomical analysis, micromorphology, and pollen, as well as a series of molecular markers (Schanzer & Kutlunina, 2010). These research tools provide clarity regarding the phylogeny of the *Rosa* genus, but currently, there are no qualitative, coherent, and sufficient data to support an acceptable classification.

The present study aimed to investigate the genetic variability of *Rosa* species and cultivars using morphological traits and namely PCR-based start codon targeted (SCoT) molecular markers, with a view to determining their degree of relatedness.

MATERIALS AND METHODS

Plant material

The samples for the morphological and molecular analysis were collected from different eco-geographic areas in order to assess their morphological and genetic diversity.

Three sources of the *Rosa canina* species were used, one from the Salina Turda area (46.5862°N 23.7861°E), Cluj County (denominated *R. canina* 1), the second from the Stațiunea Muntele Băișorii (46°33′37″N 23°20′53″E) Cluj county, (denominated *R. canina* 2) and one belonging to the ‘Can’ variety.

From the *Rosa rugosa* species, three provenances were collected. The first from the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (denominated *R. rugosa* 1), the second from Alexandru Borza Botanical Garden Cluj-Napoca (denominated *R. rugosa* 2) and the third from the Horticultural Research Station Cluj-Napoca (denominated *R. rugosa* 3).

Two other species were also studied, *Rosa gallica* cv. Saint Nicolas and *Rosa damascena,* cv. Tuscany from the Alexandru Borza Botanical Garden Cluj-Napoca.

For morphological analyses the rosehip fruits were collected in the ripening stage V (fruit surface was red) according to Medveckienė et al., 2023. Fruit length and width (mm) were measured using a digital calliper.

DNA extraction

The isolation of DNA from leaves was based on the CTAB (Cetyltrimethylammonium bromide) method using the protocol published by Lodhi et al., 1994, and improved by Pop et al., 2003 and Bodea et al., 2016.

Quantification of extracted DNA was accomplished using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and determined the quantity (ng/µl) and purity (ratio 260/280 nm) of each sample.

PCR amplification with ScoT primers

The molecular markers SCoT (Start Codon Targeted polymorphism) used in this study are based on the amplification of conservative and specific regions that flank the start codon (ATG) of the methionine amino acid on both sides. This codon is located in plant genes. The working protocol used was published by Collard & Mackill (2009), using the SuperCycler Trinity PCR thermocycler (Kyratec, Australia).

SCoT markers are a type of dominant marker that uses a single primer for PCR amplification. These markers are useful for detecting polymorphisms in plant genomes, due to their ability to target gene-rich regions.

The separation of amplification products was achieved through electrophoresis using a 1.6% agarose gel (Promega, USA) stained with a solution called RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotech, South Korea).

DNA band gels were visualized using the UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Germany). The TotalLab TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) was used to determine the number and size of DNA bands amplified using the SCoT technique.

Table 1. The SCoT primers used for the assessment of genetic relationships between *Rosa* sp. and cultivars

No.	Primer	Nucleotide sequence 5'-3' of the primer
1	SCoT 1	CAACAATGGCTACCACCA
2	SCoT 2	CAACAATGGCTACCACCC
3	SCoT 3	CAACAATGGCTACCACCG
4	SCoT 5	CAACAATGGCTACCACGA
5	SCoT 6	CAACAATGGCTACCACGC
6	SCoT 7	CAACAATGGCTACCACGG
7	SCoT 8	CAACAATGGCTACCACGT
8	SCoT 9	CAACAATGGCTACCAGCA
9	SCoT 10	CAACAATGGCTACCAGCC
10	SCoT 11	AAGCAATGGCTACCACCA
11	SCoT 12	ACGACATGGCGACCAACG
12	SCoT 16	ACCATGGCTACCACCGAC
12	SCoT 16	ACCATGGCTACCACCGAC
13	SCoT 18	ACCATGGCTACCACCGCC
14	SCoT 19	ACCATGGCTACCACCGGC
15	SCoT 21	ACGACATGGCGACCCACA
16	SCoT 22	AACCATGGCTACCACCAC
17	SCoT 25	ACGACATGGCGACCGCGA
18	SCoT 26	ACGACATGGCGACCACGT

Statistical analysis

The morphological collected data were statistically analysed using IBM SPSS Statistics 19 software. Using the Shapiro-Wilk test, the groups' normality was confirmed. The medians and percentiles (25-50-75) for non-normally distributed data were established in combination with the mean values and standard deviations for the regularly distributed data for the descriptive statistics. To do multiple comparisons, one-way ANOVA was utilized together with a post hoc Duncan test. Multivariate analysis of the data was performed using the Paleontological Statistics software (PAST) 4.11 analysis program (Hammer et al. 2001).

RESULTS AND DISCUSSION

Mophological analysis

Previous studies in *Rosa* genus (Tomljenović et al., 2022; Ercisli and Esitken, 2004) indicate the existence of variability in morphological characteristics. This provides a suitable basis for the selection process for high-quality genotypes between species of this genus.

In the present study three important morphological characteristics for eight genotypes were analyzed.

Regarding the diameter of the rosehip fruit, the lowest average value was recorded at *R. canina* Baisoara (16.46 mm), while at *R. rugosa* UASVM Cluj-Napoca was recorded the highest value (26.51 mm) with statistically significant differences (Figure 1).

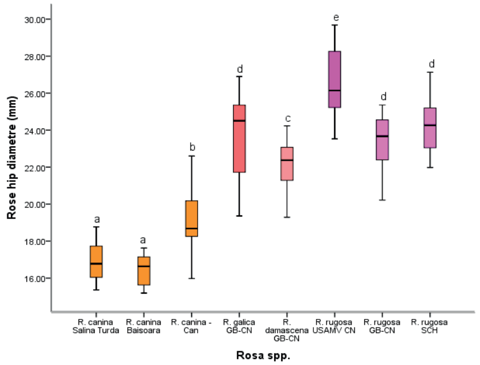

Figure 1. Rosehip fruits diameter of the genotypes analysed in the four species of *Rosa*. Each boxplot represents maximum, upper quartile, median, lower quartile and minimum values. Different letters between boxplots indicate statistically significant differences for the analysed trait, at a significance level of p < 0.05 (Duncan test)

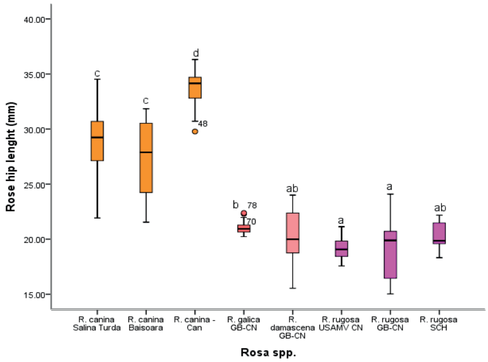

Figure 2. Rosehip fruits length of the genotypes analysed in the four species of *Rosa*. Each boxplot represents maximum, upper quartile, median, lower quartile and minimum values. Different letters between boxplots indicate statistically significant differences for the analysed trait, at a significance level of p < 0.05 (Duncan test)

Within the *R. canina* species, the 'Can' variety had the largest average diameter of the fruit (19.22 mm).

Similar results in rosehip fruit diameter were obtained by Ghiorghita et al. (2012) in a study of genotypes from local populations of *R. canina* (10.92-16.40 mm). Ercisli and Esitken (2004) following a study conducted in the province of Erzum in Turkey found values (15.04-19.69 mm) close to those recorded in our country, in the case of varieties of *R. canina*.

Within the *R. rugosa* genotypes, the highest value, statistically ensured, was obtained in the UASVM Cluj-Napoca genotype, while in the other two genotypes there were no significant differences (Figure 1).

The second morphological character analyzed was the length of rosehip fruit. In this case between *R. canina* genotypes, 'Can' cultivars be significantly different from other *R. canina* genotypes (Figure 2). The amplitude of the variation of rosehips fruits length ranged between 33.73 - 27.28 mm for *R. canina* and 19.15-20.24 mm for *R. rugosa*. The results of Duncan's multiple range tests showed that the genotype means of *R. rugosa*, *R. gallica*, and *R. damascena* are substantially lower than the mean genotypes of *R. canina*. Furthermore, it is evident that the *R. canina* 'Can' genotype's mean fruit length is decreased by a number that the boxplot identifies as an outlier (Figure 2).

According to other studies (Ghiorghita et al., 2012) the rosehip fruits length in *R. canina* genotypes varied between 16.49-25.83 mm and between 25.44-33.36 mm according to the study conducted by Ercisli and Esitken (2004). In the study conducted by Soare et al. (2015), the fruit length significantly varied among evaluated *R. canina* varieties collected from different regions of Dolj and Olt with altitudes ranging from 148 and 191 m (Soare et al., 2015). Thus, the highest fruit length has been noticed in the samples collected from the regions of Dolj, namely Șimnic and Carcea. Conversely, low values have been noticed in the regions of Olt. Although the fruits collected from Dolj regions presented the highest values in fruits length, significantly lower values have been observed in the fruits collected from the Argetoaia Dolj County. Significant differences have been observed in selected rosehip species and cultivars based on their morphological parameters. It has been revealed that *R. canina* fruits presented moderate values in length (21.5 mm) and width (16.0 mm). Similar values have been observed in *R. rugosa* fruits in the case of length and width with values of with 15.9 mm and 15.9 mm, respectively (Cunja et al., 2016). In a different study, the rosehips of *R. canina* and *R. corymbifera* presented an elongated spheroid and/or oval form. *R. sempervirens* exhibited the lowest values in width (11.8 mm), whereas *R. canina*, *R. corymbifera*, and *R. micrantha* were revealed to have significantly higher values with an average of 18.8 mm. The hips of *R. corymbifera*, *R. micrantha*, and *R. sempervirens* had an average shape index of 1.26, suggesting a spherical shape; however, the hips of *R. canina* pseudo-fruits had an index value of 1.64, which corresponds to a higher elongation (Fascella et al., 2019). The pseudofruits collected from the Northeastern region of Romania revealed significant differences in fruit shape and dimensions. Thus, the fruits' morphological parameters varied between 16.2 mm and 8.8 mm regarding width in the case of *R. corymbifera* and *R. micrantha,* both collected from the Suceava county with an altitude of ~ 500 m. Regarding the fruits' length an average value between 24.0 and 12.6 has been observed in the samples of *R. canina* collected from the Iași region (400 m altitude) and in the sample of *R. caryophyllacea* collected from Suceava county (614 m altitude) (Rosu et al., 2011).

Rosehip fruits collected in the full maturity stage from the Eastern region of Poland over the course of three years revealed significant differences in morphological parameters. Thus, the study revealed that not only there are differences among species and their geographical regions, but also between the years of collection. The highest shape indexes were recorded in 2019 and 2021 with values between 2.09 and 1.94, as revealed by the highest value in fruit length (27.9-26.9 mm) and width (13.9-13.4 mm), indicating a rounder shape. Conversely, lower values in fruit width and length have been recorded in samples collected in 2020. These differences may be influenced by several factors, including genotype, variety, environmental conditions (*i.e.*, fluctuations in temperature, precipitations, sunlight duration and intensity), region, growth conditions, harvesting period, and maturation stages (Szmagara et al., 2023).

Another morphological character analysed in this research was the number of seeds/rosehip fruit.

In this case, according to the statistical analysis it can be observed that the highest average number of seeds was registered in *R. canina* Salina Turda genotype (30.05) and the lowest average number of seeds was found in *R. rugosa* UASVM Cluj-Napoca genotype (17.15). Also, in this case, according to Figure 3, genotype *R. rugosa* UASVM Cluj-Napoca the average mean of number of seeds/rosehip fruit decreased by two numbers, identified in the boxplot as an 'outlier'.

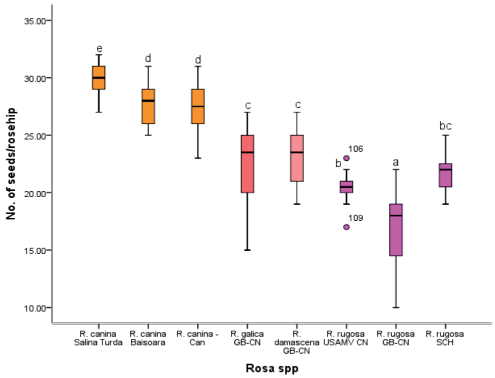

Figure 3. Number of seeds/rosehip fruit of the genotypes analysed in the four species of Rosa. Each boxplot displayed maximum, upper quartile, median, lower quartile and minimum values. Different letters between boxplots indicate statistically significant differences for the analysed trait, at a significance level of $p < 0.05$ (Duncan's test)

Gunes (2010) revealed that the average number of seeds/rosehip fruit was between 12.8 and 35.6 in a natural population of *Rosa* in northern Anatolia, Turkey. In a different study, significant differences have been noticed in seed number among and between *Rosa* sp. Thus, the highest number of seeds have been recorded in *R. gallica* genotypes (27-35), followed by *R. dumallis* (21-28). Regarding the *R. canina* genotypes, high variations in seed number (17-32) have been seen which may be due to genotype, cultivar, harvesting region, soil characteristics, altitude, harvesting time and maturation stage (Ercişli & Eşitken, 2004; Ipek & Balta, 2020).

Multivariate analysis (hierarchical clustering using paired group UPGMA, Euclidean similarity index) performed with the mean values of all morphological parameters highlights the relationships both for the *Rosa* spp. (column dendrogram) and for the distance of the three analysed traits (row dendrogram), which is also reflected in the heatmap in Figure 4.

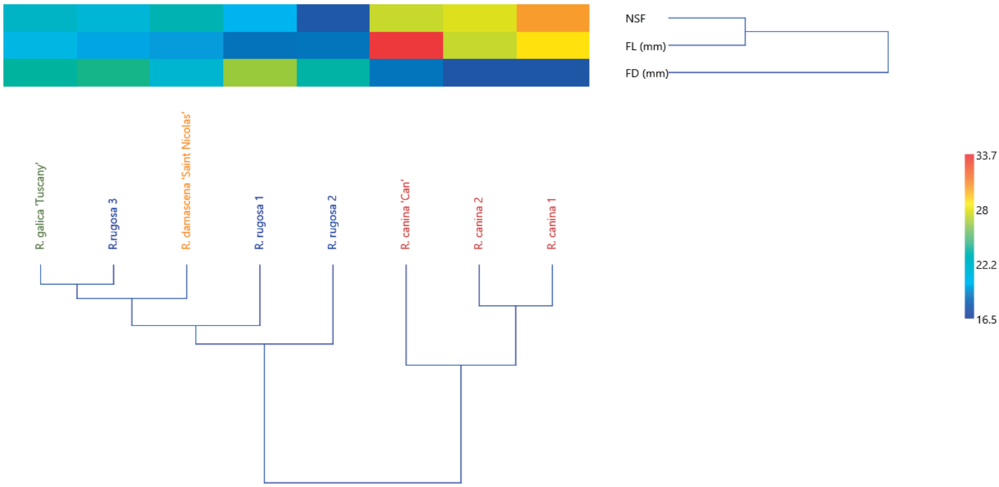

Figure 4. Two-way UPGMA dendrogram based on morphological traits, showing the relationships between the *Rosa* spp. genotypes and based on the Euclidean distance index

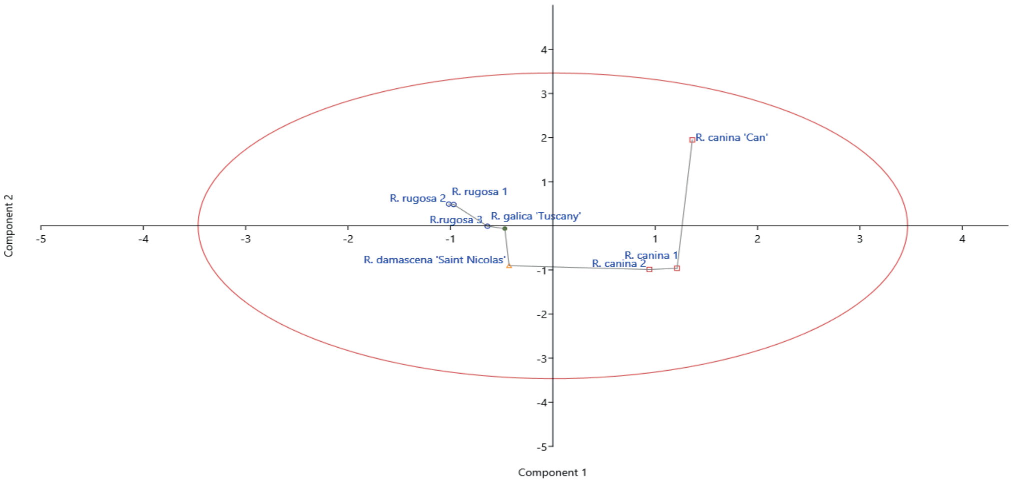

Figure 5. Principal component analysis (PCA) based on the analysed morphological traits of the genotypes of *Rosa* spp.

Thus, the grouping pattern of the eight genotypes revealed two major clusters marked as A and B (Figure 5). The first cluster of two-way dendrogram (marked as A) grouped *R. gallica* cv. 'Tuscany', *R. damascena* cv. 'Saint Nicolas' and *R. rugosa* with different provenances (*R. rugosa* UASMV-CN; *R. rugosa* GB-CN; *R. rugosa* SCH) which were discriminated mainly based on the number of seeds/rosehip fruits (NSF) and rosehip fruits diameter (FD) as shown in Figure 5. The second main cluster (B) was composed of two subgroups: one included *R. canina* cv. 'Can' and the other one with *R. canina* from two different provenances, Băişoara and Salina Turda, respectively. These subgroups were discriminated according to the mean values of rosehip fruit length (FL) and diameter (FD). The results of this study are in agreement with those reported by Singh et al. (2017) who stated that cluster analysis can be used as a valuable tool for genetic discrimination of wild roses.

Moreover, wild roses are important sources of valuable germplasm for creating variability and improving roses for future economic needs such as food, cosmetics, and pharmaceuticals (Uggla & Martinsson, 2005; Singh et al., 2020). In this study, the results of PCA of the analysed morphological traits indicated that the first three principal components (PCs) accounted for 95.91% of the total variation (data not shown). According to Reim et al. (2012), such results indicate a high level of morphological variation, suggesting that in terms of the genetic characterization of wild *Rosa* spp., evaluation of different morphological traits is still necessary. It is worth noting that, the PCA results of this study confirmed in a high proportion the grouping of *Rosa* genotypes based on UPGMA cluster analysis (Figure 4 and Figure 5).

Molecular analysis

Some previous scientific reports highlight that morphological traits have been used for germplasm characterization (Veasey et al., 2001; Rakonjac et al., 2010), but morphological traits alone may not be sufficient to determine the relationship between species (Llyod et al., 1992). Combining morphological descriptors with molecular markers could enable a more precise and efficient characterization of rose genotypes. Among DNA-based molecular markers, start codon targeted markers (SCoT) have been used to study genetic diversity in *Rosa* genotypes (Agarwal et al., 2019). The results of our study showed that these molecular markers have demonstrated their ability to reveal the genetic relationships between the analysed *Rosa* genotypes.

Of the 18 primers tested, only 12 generated amplification products for all eight samples analysed (Table 2). Six primers (SCoT 3, SCoT 7, SCoT 9, SCoT 11, SCoT 21, SCoT 22) did not generate scorable bands for all 8 samples and were therefore not included in this study. PCR amplifications with the 12 used primers were performed in duplicate to ensure the validity of the results. As an example, Figure 6 shows the electrophoretic profile obtained after PCR amplification with primer SCoT 2.

Table 2. The level of polymorphism detected with ScoT primers in *Rosa* spp.

Primer name	**Total number of bands generated/ primer**	**No. of polymorphic bands generated/ primer**	**Percentage of polymorphism (%)**	**Size range (bp) of the generated bands**
SCoT 1	7	5	71.42	435-1765
SCoT 2	11	9	81.81	460-1890
SCoT 5	10	8	80.00	345-1660
SCoT 6	12	9	75.00	385-2680
SCoT 8	8	5	62.50	330-1595
SCoT 10	11	8	72.72	360-1700
SCoT 12	12	9	75.00	245-1580
SCoT 16	9	6	66.66	320-1600
SCoT 18	13	10	76.92	285-2150
SCoT 19	9	6	66.66	315-2250
SCoT 25	15	12	80.00	245-3050
SCoT 26	13	10	76.92	435-2050
Total	130	97	-	-

In this study cluster analysis was used to assess the genetic relationships between eight *Rosa* genotypes. The built UPGMA dendrogram is based on the clustering of the recorded SCoT data and uses Euclidean genetic distances with the bootstrapping value set to 10000. The value of the cophenetic coefficient was 0.9359, which indicates a very good correlation between the original binary matrix generated from the analysis of agarose gels and the values obtained in the genetic distance matrix used for the construction of the dendrogram that expresses the genetic relationships between the four *Rosa* species analyzed with SCoT molecular markers.

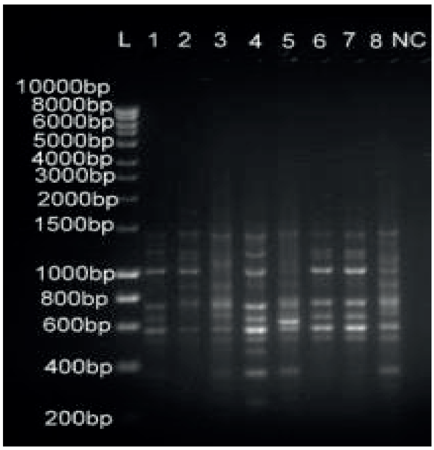

Figure 6. The electrophoretic profile obtained after PCR amplification with the primer SCoT 2. L-1Kb ladder (Bioline); 1 - *Rosa canina* 1; 2 - *Rosa canina* 2; 3 - *Rosa canina* cv. 'Can'; 4 - *Rosa damascena* cv. 'Saint Nicolas'; 5 - *Rosa gallica* cv. 'Tuscany', 6 - *Rosa rugosa* 1; 7 - *Rosa rugosa* 2; 8 - *Rosa rugosa* 3

Cluster Analysis

In Figure 7 it can be observed that the dendrogram obtained from the cluster analysis groups the eight analysed samples into two main groups (clusters): the first cluster is composed of three species, respectively *R. gallica*, *R. rugosa* and *R. damascena*, while the second group is made up of *R. canina*. It should be noted that there are differences between the samples from each subgroup, due to the different provenances of the samples within the same species, but also to the morphological and genetic differences between the species grouped in the same subgroup. These differences provide interesting data regarding the possibility of exploiting the existing genetic variability and the use of this biological material for decorative, medicinal and cosmetic purposes.

Principal coordinate analysis (PCoA) was used to validate the veracity of the results obtained from the cluster analysis. Thus, the plot generated by the PAST 4.11 program and based on the Euclidean similarity index using c = 2 as transformation exponent, shows the fact that the eight samples analysed and coming from four different species of *Rosa* are grouped axially based on 2 main coordinates that have the highest Eigen values recorded after ranking of the main 7 components, respectively axis 1 with a value of 47.123 (37.362%) and axis 2 with a value of 22.036 (17.472%).

In Figure 8, it can be seen that the three analysed species, respectively *R. damascena*, *R. rugosa* and *R. gallica*, are grouped in the 1st and 2nd Cartesian quadrants, while the samples from the species *R. canina* are grouped close, in the 3rd Cartesian quadrant. As a conclusion, the results obtained regarding the cluster analysis are also confirmed by the analysis of the main coordinates, highlighting the grouping of the data in the PCoA plot in a similar way to that in the UPGMA dendrogram, in both cases the Euclidean index of genetic dissimilarity was used.

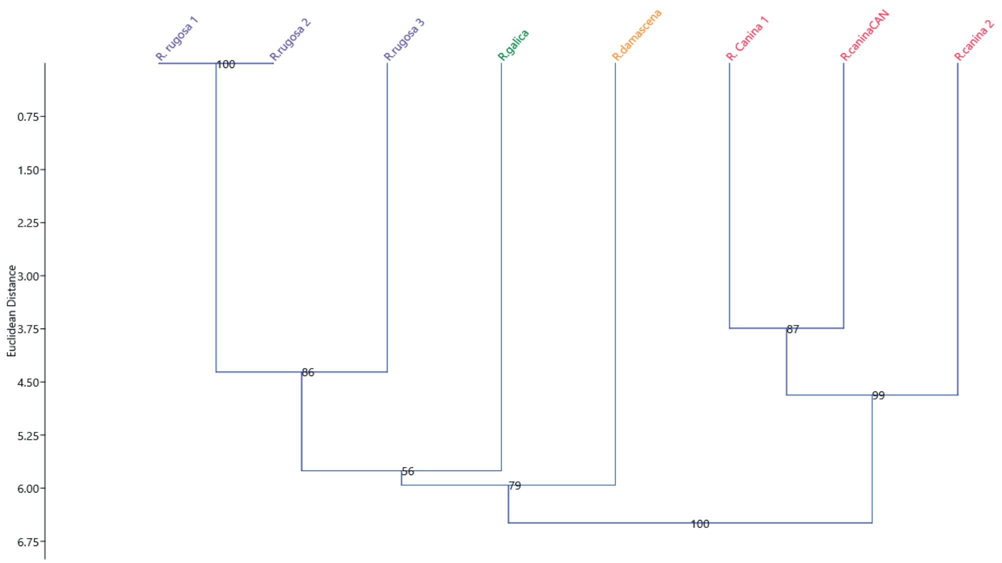

Figure 7. UPGMA dendrogram based on SCoT molecular markers analysis, showing the relationships between the *Rosa* spp. genotypes and based on the Euclidean distance index

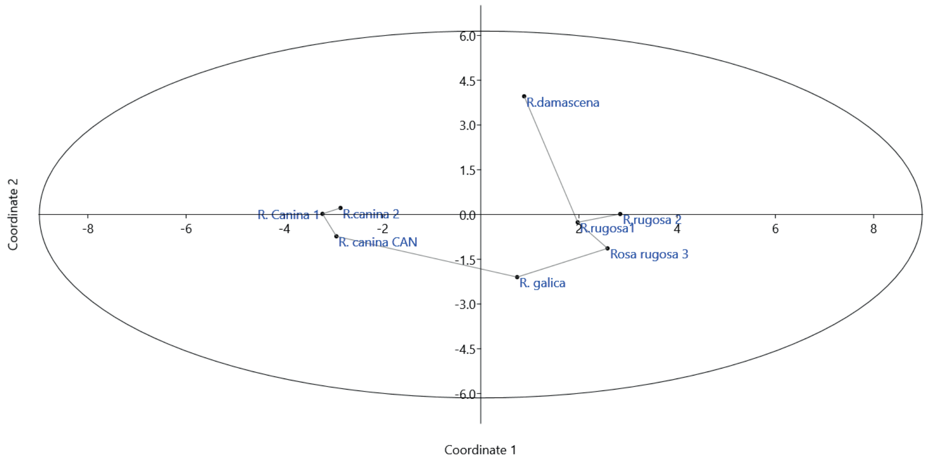

Figure 8. Principal coordinate analysis (PCoA) based on SCoT molecular markers analysis of *Rosa* spp.

CONCLUSIONS

The findings of this study revealed that some *Rosa* genotypes demonstrate the complex nature of the inheritance of some morphological traits, as seen by their significant differences in fruits length and diameter, but also the number of seeds per fruit. The assessment of genetic diversity using SCoT molecular markers provided valuable information for the genetic characterization of *Rosa* genotypes with different provenances.

The cluster analysis divided the analyzed samples into two main clusters: the first cluster comprised three species, namely *R. galica, R. rugosa, and R. damascena,* while the second group consisted of *R. canina*. It is evident that there are significant variations among the samples in each subgroup that may be attributed to the samples various origins within the same species as well as to both morphological and genetic differences between the species comprised in the same subgroup.

These results can be exploited in the near future to use the genetic potential of these genotypes for the improvement of traits required for adaptation to different environmental conditions.

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EFFECT OF ULTRASOND TREATMENT ON THE SEEDS OF DIFFERENT GLADIOLUS (*GLADIOLUS HYBRIDUS* L.) VARIETIES ON THE SOWING QUALITY

Valentin PANCHEV

Agricultural University - Plovdiv, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: valentin_panchev@abv.bg

Abstract

The main aim of the present study was to investigate the possibility of the application of ultrasound for stimulating the viability behaviors of gladiolus seeds. The studies were carried out with seeds of three varieties of gladiolus. Seeds were treated with ultrasound for 2, 4, 6, and 8 min. Germination energy, germination, main germination time, uniformity of germination, time of 50% germinated seeds, seedling morphology- length of embryo root, length of hypocotyls and fresh weight were reported. The correlation dependences on the seedling's weight with the length of embryo root and hypocotyls, as well as a linear polynomial regression between the treatment period and germination, characterized with high determination coefficients were established. The highest viability was reported in the treatment of 6 minutes of ultrasound, approximately 20% above the control. These results can be used in hybridization breeding to accelerate seed development in the propagation of corms.

Key words: *germination, gladiolus, stimulation, seedling morphology, viability.*

INTRODUCTION

Gladiolus is one of the main ornamental crops, which ranks fourth in importance in world trade (Farhat T., 2004). The genus *Gladiolus* belongs to the family *Iridaceae* (Raj et al., 2009; Azim et al., 2019) and according to some authors, such as Goldblatt. & Manning, (1998), about 255 species are currently known in this genus. The plant is native to the region of southern and tropical Africa, Madagascar and Eurasia (Takatsu et al., 2001). The species is grown mainly for cut flowers and less often for landscaping (Manning and Peter, 2008; Memon et al., 2013). Its valuable decorative properties are related to the beautiful flowers, a wide diversity of colours and upright flower-bearing stems, as well as the wonderful aroma and long vase life (Azim, 2019).

Most modern cultivars of *Gladiolus x grandiflora* are the result of selection and hybridization between six species (Barnard, 1972). At the moment, the main research activity is aimed at creating new varieties with higher decorative qualities, more diverse forms and colouring of flowers. In modern breeding, hybridization, both between different cultivars and interspecies, is a major method and tool to solve these tasks and to increase the variety of forms and cultivars (Hort et al., 2012). Gladiolus cultivars used are complex hybrids between different species (Huxley et al., 1992). After carrying out the crosses in the hybridization, the selection work continues with obtaining seeds and reproduction of the offspring from them. However, the production of high-yielding hybrid gladiolus seeds and their utilization, that is important about hybrid selection, is not always an easy task and successful (Takatsu et al., 2005). Production of gladiolus plants by seed is a relatively long period spanning several growing seasons (Bose et al., 2003; Ramzan et al., 2010).

The obtained seeds during hybridization are a relatively limited number, not always with high germination. Therefore, current science is directed toward researching various methods and ways to improve their vitality and reduce germination time (Basra et al., 2003; Ramzan et al., 2010). In this regard, Carpenter et al. (1991) reported that it is best to store the obtained hybrid seeds of gladiolus, intending to maintain good sowing qualities, due to the relatively long period between seed collection and sowing, at a temperature in the range of 5 to 15°C. Improvement of germination processes of gladiolus seeds Ramzan et al. (2010) achieved by pre-treatment of the gladiolus seeds with potassium nitrate (KNO_3), reaching a germination rate of 92%. Good results in the treatment of gladiolus seeds, also with potassium nitrate, were also reported by Memon et al. (2013). In evaluating gladiolus seed priming Mushtaq et al. (2012) established a significant improvement in germination.

There are relatively few scientific reports on the application of different physical methods of impact to improve the vitality and the sowing qualities of gladiolus seeds. Carpenter et al. (1991) studied the effect of temperature and air humidity on the gladiolus seeds viability status. There is almost no research on the effect of ultrasound treatment on seeds of this species. Aladjadjiyan (2007) emphasized that the application of ultrasound is an extremely promising and effective way to improve the vital status of seeds.

The main goal of the present study is to investigate the influence of ultrasound treatment on the vitality behaviours of gladiolus seeds, with the aim of accelerating their development.

MATERIALS AND METHODS

The experiments were carried out in the Experimental field and Scientific laboratory of the Department of Horticulture at the Agricultural University - Plovdiv, Bulgaria. Seeds of the following gladiolus cultivars Purple flora, Oscar, and Plum tart, were produced by growing the plants through corms. After flowering and fruit formation, in at full botanical maturity stage, the fruits were harvested, the seeds were extracted and they have been stored under laboratory conditions in paper pouches. Three months after the collection of the seed, they were treated with ultrasound in the following variants:

1. Control (not treated seeds); 2. Ultrasound -2 min; 3. Ultrasound - 4 min; 4. Ultrasound - 6 min; 5.Ultrasound - 8 min.

The treatment has been completed with an ultrasonic bath Nahita, model 620-1 of the company Auxilab, S.L. from Spain with the following parameters: frequency 40 KHz, volume 0.6 l.

After treatment of the seeds, their vital signs were evaluated. Seeds were placed in 10 cm diameter Petri dishes with Watman 1 filter paper moistened with 5 ml of water. Germination energy and germination were determined, according to the requirements of ISTA (2013) and under the recommendations in the manner described by (Ramzan et al., 2010), as 100 seeds were put in four replicates. The time to germination of 50% of the seeds (T50) was determined according to Farooq (2005). Mean germination time (MGT), described by Kader (2005) and Uniformity of germination (described by Panayotov, 2015) were calculated. When determining germination, the weight of the sprouts was established from all germinated seeds in the 4 repetitions and recalculated for one sprout. At this stage, the embryo root length and hypocotyls length of ten seedlings from the 4 replicates were measured.

Data were subjected to ANOVA analysis of variance, correlation and regression analyses (Fowel and Cohen, 1992).

RESULTS AND DISCUSSIONS

The influence of different methods and ways of pre-sowing impacts on crops is established very well by studying their sowing behaviours. One of the important characteristics in this scope is the germination energy. Its values are established for a shorter period, which gives reason to consider these earlier germinated seeds that are with higher vitality (Panayotov, 2015). The effect of different durations of exposure to ultrasound is very well demonstrated (Table 1). A strong varietal reaction is also observed. Sonication for up to 6 min produced a gradual increase in seed germination compared to the control, except for Plum tart. This tendency is most strongly observed in the Purple flora variety, where the difference with untreated seeds reaches 20%. A similar state is reported for the Oscar variety, but with a lower difference of 12.67%. However, the effect on Plum tart seeds was the strongest at 2 min of sonication, with an increase of 10%, after which it decreased, but the values were higher than those of control seeds. A strong pronounced inhibition in all three varieties is established at the 8-minute variant, with a decrease, compared to the control, between 16.67% (Plum tart) and 23.33% (Purple flora).

Table 1. Sowing parameters of gladiolus seed after application of ultrasound

№	Variants	Germination energy (%)			Germination (%)			Time of sprouting of 50% of seeds (T_{50}) (%)		
		Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart
1	Control	46.66	40.00	46.66	73.33	83.33	70.00	7.1	7.3	6.9
2	2 min	53.33	43.33	56.66	76.66	86.66	73.33	6.7	7.2	5.6
3	4 min	56.66	43.33	50.00	76.66	66.66	83.33	6.4	6.3	5.9
4	6 min	66.66	53.33	50.00	93.33	66.66	90.00	6.3	6.3	6.7
5	8 min	23.33	23.33	26.66	46.66	50.00	53.33	8.2	9.0	8.6
LSD p = 0.05		11.2	6.4	8.1	11.2	14.3	9.1	1.4	1.8	1.0

The vital status of seeds is almost fully determined using germination. The values of this indicator fully reflect the quality of the seeds and are used to standardize their sowing and agronomic value (Black et al., 2006). A very pronounced variety response is also observed for this characteristic. This parameter increases uniformity to sonication at 6 minutes was found in Purple flora and Plum tart, with germination reaching 93.33% and 90.0%, respectively, or 20% more than untreated seeds. For Oscar, the highest values were observed in variant 2 minutes, and the increase compared to the control was significantly smaller - by 3.33%. In this variety, in the remaining two periods of ultrasound application, a reduction compared to the control was observed. A very strong suppressive effect, as for germinating energy, was reported at 8 minutes for all three studied varieties. Compared to the control, the reduction was 16.67% for Plum tart to 33.33% for Oscar. The data are mathematically proven except for germination for the three cultivars and germination energy for Oscar. Aladjadjiyan (2007) points out that ultrasound, representing mechanical waves of high frequency, which in plant organism, including seeds, cause an increase in molecular energy and changes in the structure of substances. According to this author, the sonication of seeds for 5 minutes there is a strong effect and increases the germination energy and germination (Aladjadjiyan, 2007a). Similar conclusions were reported by Miano et al. (2015) and Patero and Augusto (2015). Stimulating influences of ultrasound on the germination of seeds of ornamental and flower crops and its uniform change depending on the duration of exposure were reported also by Panchev (2022). The above-mentioned tendency for changes in germination depending on the duration of exposure to ultrasound is complemented very successfully through the established regression relationships (Figure 1). The type of regression in all three varieties is polynomial with high coefficients of determination R^2 = 0.55, R^2 = 0.89 and R^2 = 0.94, respectively for Purple flora, Oscar and Plum tart. Through these coefficients, is possible to determine how the factorial variable affects the dispersion percentage of the resulting variable. Correspondingly between 55% and 94% of the cases, sonication will produce the indicated changes in the germination of the gladiolus seeds.

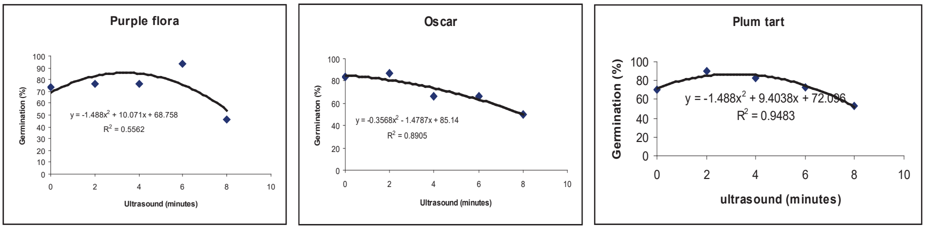

Figure 1. Regression dependence of germination and the duration of ultrasound treatment of gladiolus seeds

The time for germination of 50% (T50) of gladiolus seeds under the influence of ultrasound changes in a narrow range. This time for controlling seeds is approximately 7 days. In the varieties Purple flora and Oscar, it is the shortest at sonication of 6 minutes, and in the first two durations the effect is weaker, but the results are better than those of untreated seeds. Differences from the above-mentioned are observed for Plum tart. The least days for germination of 50% of the seeds of this cultivar were under ultrasound for 2 minutes, after which it increased but was still better than the control. Seed germination was accelerated. As with germination, the highest treatment duration of 8 minutes resulted in a high deterioration, this time increasing approximately between 15% and 25% depending on the variety.

Mean germination time (Table 2) is an essential indicator for evaluating the sowing and viable quality of the seeds and indicates the average number of days that were needed for one seed to germinate (Traynov, 2021). The most as a result of the application of ultrasound for 6 minutes, and depending on the variety, the improvement compared to the control was between 10.7% (Purple flora) and 31.03% (Plum tart). Duration of 2 and 4 minutes also have a stimulating effect on this trait, although weaker. A strong inhibitory effect was found at 8 minutes, with the increase over control being greater by 20.8% to 34.21% for Purple flora and Plum tart, respectively. Uniformity of germination provides significant information about the behaviour of seeds sowing under the influence of a given impact on them. An increase in the uniformity of germination was observed at all tested durations, except for 8 minutes. The best results for the varieties Purple flora and Plum tart were recorded at 6 minutes of ultrasound, and for Oscar - at 4 minutes, approximately 7% above the untreated seeds. The strongest influence was found for Purple flora seeds. As with the other signs discussed above, a pronounced inhibition was present after exposure by 8 minutes, with values decreasing by approximately 30% than to the control.

Table 2. Sowing parameters of gladiolus seeds under the effect of ultrasound application (days)

№	Variant	Mean germination time (days)			Uniformity of germination (%)		
		Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart
1	Control	7.2	7.5	7.6	13.3	11.7	12.4
2	2 min	6.7	7.3	7.0	18.3	16.7	14.4
3	4 min	6.5	7.0	6.3	20.1	18.9	15.1
4	6 min	6.5	6.3	5.8	20.9	16.1	18.9
5	8 min	8.7	9.8	10.2	9.7	8.8	9.3
LSD p =0.05%		1.6	1.0	1.3	3.8	5.2	3.2

Treatment of the seeds with ultrasound causes changes in the morphological characteristics of the sprouts (Table 3). The length of the embryo root compared to the control increased for Oscar and Plum tart varieties up to 6 minutes of treatment and reached 2.59 cm and 2.65 cm, respectively, and for Purple flora it was 2.25 cm at 4 minutes. A significant reduction was found at 8 minutes of exposure, most pronounced in the variety Plum tart, with 46.22% lower than the control. These data are statistically significant. A similar trend is observed for hypocotyls length. For cultivars Purple flora and Oscar, treatment with 6 minutes increased the most length, by 19.94% and 14.89% over the control, respectively. In the other variety Plum tart, this increase is 39.80% and is at 4 minutes. Inhibition from treatment with 8 minutes was also found for this trait, with the reduction ranging from 13.85% (Plum tart) to 21.29% (Purple flora). Seedling fresh weight is a major factor in determining seed vigor (Copeland & McDonald, 2001). In the three studied varieties, it increases uniformly up to 6 minutes. The increase compared to the control was the highest for Plum tart at 41.09% and the weight reached 41.2 mg. It was lower compared to untreated seeds for all varieties in the 8-minute treatment. A strong positive correlation was found between seedling fresh weight and embryo root and hypocotyls length, except for hypocotyls length in Plum tart, which was also positive but middle.

Table 3. Morphological characteristics of gladiolus seedlings after application of ultrasound

№	Variants	Length of embryo root (cm)			Length of hypocotyls (cm)			Fresh weight of one seedling (mg)		
		Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart
1	Control	1.87	1.43	1.55	3.76	2.47	3.04	37.1	30.0	29.2
2	2 min	2.19	1.47	2.28	3.90	3.42	3.25	37.2	31.1	33.6
3	4 min	2.25	2.14	2.13	4.11	3.67	4.25	38.6	32.8	33.6
4	6 min	2.04	2.59	2.65	4.51	4.32	3.44	41.4	35.3	41.2
5	8 min	1.34	0.96	1.06	3.10	2.16	2.67	33.5	28.2	28.4
LSD p = 0.05		0.4	0.6	0.71	0.41	1.1	0.39	1.1	2.4	2.8
r*		0.73	0.98	0.91	0.99	0.96	0.46			

r* Correlation coefficient with fresh weight of one seedling

CONCLUSIONS

The application of ultrasound to gladiolus seeds there is a pronounced effect on their sowing qualities, with a well-defined variety response observed. Germination energy and germination in most cases are increased the most from exposure to 6 minutes. Polynomial regression with high coefficients of determination was found between treatment duration and germination.

A positive influence of ultrasound treatment is reported on the overall vital status of gladiolus seeds, improving parameters such as mean germination time, uniformity of germination and the time for germination of 50% of the seeds. The development of seedlings is the best in treatment with 6 minutes. High inhibition causes ultrasound with a duration of 8 minutes.

It is recommended to apply a treatment of gladiolus seeds with 6 minutes of ultrasound. The obtained results can be predominantly used to improve seed development, especially in hybridization breeding programs. This will highly improve the research activities in hybrid selection, which will increase their effectivity.

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INNOVATIVE RESEARCH ON GERMINATION OF BASIL SEEDS (*OCIMUM* SPP.)

Dumitru-Mitel TOMA[1], Floarea BURNICHI[1], Emilian MIREA[1], Florin TOMA[2]

[1]Vegetable Research and Development Station Buzau, 23 Mesteacanului Street, Buzau, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mitel.toma25@gmail.com

Abstract

Healing with herbs is as old as mankind. The connection between man and his search for remedies in nature dates back to the distant past, of which there is ample evidence. One of the plants often used for this purpose is the basil (Ocimum spp), a plant with multiple values: biblical, medicinal, ornamental, culinary and social. In this regard, research has been conducted on the optimal method of seed germination in this species. The tests were performed at the Vegetable Research and Development Station (SCDL) Buzau. The experiment involved the establishment of eight study variants, with three repetitions each. The results are statistically assured. Following the research on the germination faculty, it was found that in the variant where aghiasma (Holy water) was used for wetting the seeds, there was the highest number of germs of the species under observation. In conclusion, by establishing the optimal method of twinning basil seeds, producers will benefit from the economic efficiency of the crop, and the finished product will reach the final consumer at a low price and high quality.

Key words: *basil, germination, Holy water, production, quality.*

INTRODUCTION

In Romanian: "Busuiocl"; in Hebrew: "Rihau"; in French: "Basil"; in English: "Basil", "Sweet basil", "Exotic basil"; in German: "Basilienkraut". Scientific name: *Ocimum basilicum* L. Family Lamiaceae. Popular name: sweet basil, borjolica, basil, mint, basil (Bojor O., Dumitru R., 2007). This plant has been cultivated for over 5000 years, so its origins are obscure. It is believed to originate from the Indian subcontinent, from there spreading to various regions of the globe (https://gradinaistorica.ro/busuioc/). Basil was cultivated in China as early as 800 BC (Enachescu G., 1984), to the east and by the Egyptians, Greeks and Romans, closer to us, and through the influence of these cultures it was extended, over time, to the less warm areas of the European continent (because it lends itself very well to growing in pots) (https://gradinaistorica.ro/busuioc/). This plant was brought to Europe by Charlemagne and was cultivated as a medicinal herb in the gardens near the monasteries. In the ancient pre-Christian cultures of the Near East, but especially in Palestine, basil was considered a holy plant, used for religious purposes, being preserved until the times of the Christianization of Europe (Borloveanu M., 2014). In our country we find it mostly in the rural area, cultivated in the gardens around the houses (Burnichi F., Vlad C., Strugariu C. G., Bebea P., 2014). In Romania, the exact period and area in which this plant was introduced for the first time is not known, but it is assumed that it would have entered our country around the century. XVI. Initially, basil was not found in organized cultures, but only sporadically, it being often used as a sacred plant in church worship. The water in which the basil is soaked acquires special properties. Phytoncides and volatile oil dissolved in water give it disinfectant, comforting, sanitizing properties (Vînătoru C., Mușat B., Bratu C., 2019). Basil is a valuable biblical, aromatic, ornamental, social and medicinal plant, widely used in both traditional and scientific medicine, in the perfume and food industry, in cosmetics, organic agriculture, as a companion plant, landscaping territory, and as a vegetable product it has a deep religious significance, serving as an object of worship. This paper aims to present in a synthetic manner the most important implications of the species *Ocimum basilicum* in human concerns of all times, highlighting the potential of its use in various fields.

Taxonomy

The genus *Ocimum* is known as one of the most famous genera in the Lamiaceae family and currently comprises more than 150 species. Distribution is mainly in tropical regions. Recently, they have been cultivated worldwide as culinary herbs and for the extraction of essential oils. Taxonomic identification within the genus and between cultivars can be done by morphological characteristics such as leaf shape and color, flower and seed morphology. Numerous polymorphisms resulting from extensive cultivation and inter- and intra-specific cross-hybridization result in a wide range of subspecies, each with its own chemical composition and biological activity. In Romanian: "Busuioc"; in Hebrew: "Rihau"; in French: "Basil"; in English: "Basil", "Sweet basil", "Exotic basil"; in German: "Basilienkraut". Scientific name: *Ocimum basilicum* L. Family *Lamiaceae*. Popular name: sweet basil, borjolica, basil, mint, basil (Bojor O., Dumitru R., 2007). The term basil comes from the Greek language, βασιλευς (basileus) meaning "king", this plant is said to have grown on the site where the Emperors Constantine and Helena discovered the Holy Cross. The Oxford English Dictionary mentions some speculation that basil was used in "some royal salves or medicines". Basil is still considered the "king of spices" by many chefs and food book authors (https://ro.wikipedia.org/wiki/Busuioc).

The Lamiaceae family is one of the families with the largest number of species cataloged as aromatic plants, the classification of the genus *Ocimum* of which busuiocum is a part, undergoing changes over time due to various discoveries and increasingly precise taxonomic studies, the systematic classification within this genus thus becoming very complex (Upson and Andrews, 2004; Moja et al., 2016). *Ocimum basillicum* plants studied in the present paper have the following distinct morphological characteristics.

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: *basilicum*
(https://www.mdpi.com/2311-7524/8/2/144/htm)

Biblical uses

Basil (*Ocimum basilicum* L.) has always been considered a sacred plant. Precisely for that reason, only the basilisk had the right to pick it, associating this gesture with a ritual full of mystery. In India, where it originates, basil was raised to the rank of "sacred plant" (tulsi), being cultivated only in specially arranged places around sanctuaries. For the ancient Greeks, basil was the "king of plants" (https://www.gustos.ro/sfaturi-culinare/sa-mancam-sanatos/tot-ce-trebuie-sa-stii-despre-busuioc.html). Basil is not mentioned in the Bible. Similar to him would be, in the Old Testament, Hyssop, with which the sacrifices and the people were sprinkled as a sign of soul cleansing; it was believed to bring good luck to a person's home. Even today, mothers in rural communities perfume the shirts of young men with basil, and girls, on the eve of the Baptism of the Lord, put basil under their pillow to dream of their lover. The essential oil of basil (Basil Oil) enters as an ingredient in the preparation of the Holy and Great Mir (Bojor O., Dumitru R., 2007). Basil is also used in the process of sanctifying water. In general, the priest immerses a cross and a basil sprig in a vessel of water, this procedure, followed by a church order, specific to the sanctification of water, causes the water to be transformed, by the descent of the Holy Spirit, into aghiasma. Also with this plant, the priest sanctifies people's houses, icons, objects of worship, cars, and last but not least, the person is sprinkled, to cleanse the body and soul. From our grandmothers we inherited the habit of asking the priest for a few sprigs of basil to be placed on the icons, to decorate and venerate them. And the Holy Sacrament of baptism is performed by sprinkling the baby with the bunch of basil soaked in water, also at this Holy Sacrament the basil is used together with the child's diaper to be sprinkled for cleansing. From the "sacred grass" to the "magical one" it was not a long way. People have invented all kinds of customs related to the power given by eating or simply touching basil. Beyond rituals and superstitions, basil is also used in Orthodox rituals (https://www.gustos.ro/sfaturi-culinare/sa-mancam-sanatos/tot-ce-trebuie-sa-stii-despre-busuioc.html).

Social uses

When it comes to the social uses of basil, we are talking about traditions and customs related to this herb. One of the most widespread customs, carried out with the help of basil, is the one that takes place on Epiphany night. That night, the young girls who wanted to dream of their bear had to tie a red silk thread and a piece of basil on their ring finger, then, as when they went to bed, after having previously fasted with a black or harsh fast, they put sprigs of basil under the pillow (https://www.crestinortodox.ro/datini-obiceiuri-superstitii/traditii-obiceiuri-boboteaza-68744.html). In the child's first bath, basil was compulsorily placed, so that the newborn would be loved and honest, as basil is, and smell nice. Another custom by which young, unmarried girls can find out their chosen one is to put basil at the well guides, and if the next day they find the basil with a prom, then they will get married that winter (Tudor P., 1914). Most often, however, basil appears in wedding customs. In the Apuseni Mountains, but also in other parts, the bride and groom's crowns were made of sasau (known in Moldova as barbănoc) and basil. When leaving the house, the dance, also called "Basil", was played, accompanied by shouts that indicate the magical function of the dance: "Three times after the meal, / May the evil leave the house, / May the good remain, / May the youth live" . When she left her parents' house, the bride held in her hand a bunch of basil wrapped in a beautifully sewn scarf, which she twirled three times above her head, so that the other girls of her age would also marry. In the north of Moldova, brides, when going to the wedding, girded themselves with basil and put it in their breasts, to enjoy the honor enjoyed by basil. In the south of the country, before the wedding, the bride went to the well with two boys, whose parents were both alive. From home to the fountain they stopped three times and played three dances. Then he took water from the well in the small pot and put basil in the water. On their way back, they also danced three times around the donut with water and basil. When the groom arrived with his groomsmen, they were sprinkled by the bride and groom with basil soaked in water and, finally, they poured the water on their feet. Basil was placed in the pillow on which the bride and groom would sleep. In Ialomiţa county, the bride, accompanied by a boy or two and fiddlers, brought a bowl of water, decorated with basil, and placed it next to the wedding tree. Water was played around the tree, and when the groom and the bride arrived, the bride sprinkled them three times with the basil soaked in water. And the great mother-in-law, when she welcomed her daughter-in-law, among other symbolic objects, also had a sprig of basil (Costin Costin C., 2021).

Ornamental and landscape uses

Basil can be used fresh in floral arrangements or dried, to make various design objects (Morales M.R., Simon, J.E., 1997). Basil is a decorative, annual plant native to India. Alone or in a combination of varieties, basil is used to decorate yards and gardens. If it is grown in the garden, it can grow up to 50-60 cm high, and if it is grown in the planter, it remains shorter, 20-30 cm high (https://www.agrodenmar.ro/busuioc-rosu).

The main four decorative varieties of basil that are part of the collection of the Buzau Vegetable Research-Development Station are: *Ocimum basilicum* L. var. *violaceum* hort. *Purple Seraphim basil*, *Ocimum basilicum* L. - Basil Scented with Buzău, *Ocimum basilicum* var. *citriodorum* - Macedon lemon-flavored basil, *Ocimum basilicum* var. *minimum* L.- The 'Smarald' dwarf basil variety.

Medicinal uses

Medicinal plants are increasingly widely cultivated to meet the high demand for natural remedies. These species are generally cultivated in the open field, which results in a large variability from year to year, both in biomass production and in the content of active principles (R. Maggini, C. Kiferle, L. Guidi, A. Pardossi, A. Raffaelli, 2012). Basil (*Ocimum basilicum* L.) is an annual plant native to India that has been cultivated for several millennia for its culinary and medicinal uses (P. Pushpangadan, V. George, 2012).

Basil has traditionally been used as a medicinal herb for various purposes, such as headaches, coughs, diarrhea, constipation, intestinal worms, and kidney dysfunction. It is also considered a stomachic antispasmodic, carminative, antimalarial, febrifuge (Wome, 1982; Giron, 1991). Essential oils are a diverse group of natural products that are important sources of aromatic and flavoring chemicals in food, industrial and pharmaceutical products. Essential oils are complex mixtures of volatile compounds particularly abundant in aromatic plants, composed largely of terpenes and aromatic phenylpropanoid compounds. The essential oil composition of plants varies and is due to genetic and environmental factors. Due to their chemical composition, essential oils possess numerous biological activities (antioxidant, anti-inflammatory, antimicrobial, etc.) of great interest in the food and cosmetic industry, as well as in the field of human health. (Bernath, 1986). The essential oil content of plant tissue also varies with developmental stage (Burbott and Loomis, 1967) and may vary with extraction methods (Guenther, 1972). The chemical composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) was analyzed by gas chromatography (GC) coupled with mass spectrometry (GC-MS). The components identified constituted 99.03%, 95.04% and 97.66% in oils from flowers, leaves and stems, respectively. The main constituents of essential oils from flowers, leaves and stems were estragole (58.26%, 52.60% and 15.91%) and limonene (19.41%, 13.64% and 2.40%) and p - cumin (0.38%, 2.32% and 2.40%). Apiol (50.07%) was identified as the highest major constituent for the strain. This was followed by estragole (15.91%) and exo-phenyl acetate (6.14%). Minor qualitative and major quantitative variations for some essential oil compounds were determined in relation to different parts of *O. basilicum*. The chemical composition of different parts of basil oils has been reported to be highly variable. It is known that specific chemotypes of estragole are also known (Jean-Claude Chalchat, Mehmet Musa Özcan, 2008). Essential oils, as sources of natural products, represent an alternative to synthetic antioxidants and antimicrobial agents in the food and pharmaceutical industries, alternative medicine and natural therapy. Basil (*Ocimum basilicum* L.) belongs to the Lamiaceae Family, which, in addition to being used as a spice, is also known as a powerful antibacterial, antimutagenic and chemopreventive medicine. The antioxidant activity of the essential oil was investigated spectrophotometrically by the DPPH test and the antimicrobial activity using the agar diffusion method on the following microorganisms: *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Providencia stuulii*, *Providencia stuartii*, *Streptococcus* group D, *Salmonella* spp. and *Candida albicans*. The results obtained proved the presence of 65 components, with the highest content of linalool (31.6%) and methyl chavicol (23.8%). The essential oil showed the best antioxidant properties after 90 minutes of incubation with an EC50 value of 2.38 mg/ml. The oil showed the best coagulase-positive antimicrobial activity on staphylococcus. The chemical composition, antioxidant and antimicrobial activity of the investigated basil essential oil indicate a significant phytomedical potential (Ljiljana P. Stanojevic, Zeljka R. Marjanovic-Balaban, Vesna D. Kalaba, Jelena S. Stanojevic, Dragan J. Cvetkovic & Milorad D. Cakic , 2017).

Culinary uses

Basil is one of the most popular culinary herbs in North America (De Baggio and Belsinger, 1996). Basil is one of the most popular and useful culinary herbs due to its delicate aroma and fragrance. Cultivation methods vary depending on the part of basil used (fresh or dried leaves, essential oil, seeds, processing for industrial purposes and the climatic conditions in which it is grown. The main forms of use of the different types of basil are: fresh basil, frozen, dried, essential oil, medicinal and other uses (Raimo H., Yvonn H., 2005). In Mediterranean cuisine, basil is a highly valued spice. Fresh or dried, it is used in tomato sauces and white sauces, with which they eat pasta, pizza and some salads. Also, basil is highly appreciated, especially in Asian cuisine, as a flavoring in fruit salads, compotes and even jams. Added to salads, basil is combined with parsley leaves, mint, with finely chopped tomatoes and lettuce leaves In North Africa and the Middle East, an energy drink is prepared by putting a liter of water, four tablespoons of honey and 1-2 dried basil sprigs in a bottle. Everything is left to macerate for 12 hours, after which the obtained preparation is filtered and drunk in several installments (Tudor I., Formula AS, 2019).

MATERIALS AND METHODS

In order The four main decorative varieties of basil that are part of the collection of the Vegetable Research and Development Station Buzau are: *Ocimum basilicum* L. var. *violaceum* hort. Purple basil Seraphim, *Ocimum sanctum* Linn. - Holy basil, *Ocimum basilicum* var. *minimum* L. - 'Smarald' dwarf basil variety.
Purple basil variety 'Serafim' (Figure 1)
Species: *Ocimum basilicum L.* var. *violaceum* hort. - Semi-early variety, suitable for cultivation in the greenhouse, solarium, field, pots, for food, seasoning, ornamental-decorative; the shape of the bush is semi-erect, strongly branched.

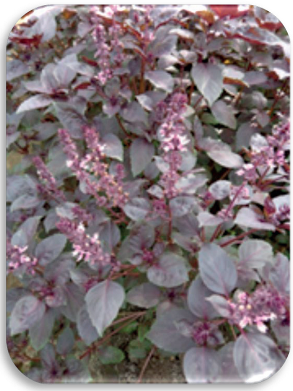

Figure 1. *Ocimum basilicum* L. var. *violaceum*

The variety is highlighted by the special color of the foliage, purple-purple, pink-purple flowers and the specific aroma, mentholated, released by all the organs of the plant, especially the leaves and inflorescences.
Plant height: 60-70 cm, medium-rich foliage, with slightly serrated leaves, purple-purple color due to the high content of anthocyanins.
Resistant to specific diseases and pests.
Production capacity: over 12 t / ha average production of shoots, fresh substance. It can be grown all year round in heated sheltered areas or in pots.
It is recommended to establish the culture by seedling. Suitable variety in organic farming. It is sown between March 1-10, for field cultivation, emerges after 6-7 days and is planted at the end of April.
It is harvested in stages, from the beginning of the bush formation until the arrival of its total frost, at the technical or physiological maturity (Burnichi F., Vlad C., Strugariu C. G., Bebea P., 2014).

'SMARALD' dwarf basil variety (Figure 2)
Ocimum basilicum L. var. *minimum* - Semi-early dwarf basil variety obtained from S.C.D.L. Buzau, approved in 2021.
Annual plant, with average plant height 20 cm, average diameter of the bush 20 cm, globular plant shape, branched, dense bush. The foliage is small - rich, with slightly sharp leaves, the shape of the leaf tongue - elliptical.
The color of the leaves is emerald green, without anthocyanin coloration on the upper side of the tongue of the leaf. The flower has a white corolla. Flowering period - with the beginning of summer. The seeds are 2/1 mm elliptical, slightly blackish brown, matte.
The mass of 1000 seeds is 1.3 g, and in one gram there are 750 seeds. Pollination is free, entomophilic. It can be successfully grown both in pots and in protected areas and in the open field.
The production of fresh grass is 6-8 t/ha.
The variety is resistant to specific diseases and pests, the crop not registering 8 losses. Average seed storage capacity (3-4 years) without diminishing the germination capacity too much.
It is an aromatic, spicy, medicinal and ornamental plant - decorative. It is cultivated by direct sowing and by seedlings, the optimal planting distance being 20 cm between plants per row and 20-30 cm between rows.
It is used in the food, cosmetics and complementary medicine industries, and there is the possibility of its use in the perfume industry due to its specific, slightly pleasant, slightly mentholated aroma. It is suitable for growing in pots, being recommended for purifying the air in the rooms.
It can be cultivated in an ecological system, being insect repellent (authors: Floarea

Burnichi, Toma Dumitru Mitel, Staicu Bogdan Gabriel, Petre Constantin) (Figure 4).

Figure 2. *Ocimum basilicum* L.var. *minimum*

Ocimum sanctum Linn. - Holy Basil (Figure 3)

Holy basil is an important medicinal and religious plant closely related to the basil we use for cooking. *Ocimum tenuiflorum* is closely related to culinary basil (*Ocimum basilicum*), but differs in being a short-lived perennial with smaller flowers. Commonly known as holy basil or tulsi and tulasi in South Asia, it is an important sacred plant in Hinduism and, as with many plant species used in Asia, the religious uses are often linked with the medicinal uses. Historically, holy basil was frequently grown in large vessels in the courtyards of Hindu forts and temples to cleanse the body. One of the plant's synonyms, *Ocimum sanctum*, reflects this religious connection.

Holy basil is an aromatic, perennial herb up to 1 m tall, sometimes purplish in colour.

The leaves are elliptic (narrow oval) in shape. The fruits have four small brown nutlets, which, unlike basil, do not produce a lot of mucilage when wet.

Figure 3. *Ocimum sanctum* Linn. - Holy Basil

Nr. crt.	Period	Work	Execution mode		Remarks
			Quantities per hectare	Used	
1	oct. – nov.	land preparation	- discussed the previous culture; - basic fertilization with phosphorus 40-50 kg / ha s.a. and potassium 30-40 kg / ha s .; - plowing 28-30 cm deep	- disc harrow G.D.-3.2; - M.A. amendment spreader. –3.5; - PRN-3 reversible plow	
2	mar. – apr.	preparation of the germination bed	- fertilized with 40 kg nitrogen, etc .; - discussed; - modeling the ground in raised furrows with a crown width of 104 cm + sowing - 4 kg of seed	- M.A. amendment spreader. –3.5; - disc harrow G.D.-3.2; - multifunctional aggregate 1.4 - made at S.C.D.L. Buzau, who performs both works in a single pass	- sowing scheme 11 + 18 + 18 + 18 + 11 x 4 15 cm => 250000 pl. / ha Sowing is done when the average daily temperatures are 15 oC
3	apr. – mai	thinning	- when the plants are 6-8 cm tall, thinning is done, which coincides with the first manual pruning	- manual	
4	apr. – aug.	Watering	- 300-400 m3 of water, 4-5 times	- manually, on long furrows	
5	apr. - aug	hoeing	- 4-5 times, to restore the gutters after irrigation	- vegetable grower C.L. –1.4	- watering, aerating, phytosanitary treatments, fertilizers
6	apr. – aug.	prăşit	- de 2-3 ori pe rând	- manual	
7	mai – aug.	disease and pest control	- treatments with specific pesticides	- M.P.S.P.-3.3 spraying and dusting machine	*- against: cercosporiosis, root rot, wireworm. It is good to avoid the attack of diseases and pests through cultural hygiene, higher agrotechnics, respect for crop rotation*
8	aug. - sept.	harvesting	- 1000-1200 kg of fresh grass	- manual, with sickle	- harvesting is done at the beginning of the opening of the flowers on the main inflorescence, after 10 o'clock, in clear weather

Figure 4. Basil cultivation technology sheet - used at S.C.D.L Buzău

The experiment involved performing a germination test with eight study variants, in three repetitions for each variety studied, differing as follows:

V1 - Water (control);

V2 - Infusion (concentration 25 g plant/ 250 ml water);

V3 - Decoction (concentration 100 g plant/1000 ml water);

V4 - Infusion (concentration 25 g plant/ 500 ml water);

V5 - Decoction (100 g plant/2000 ml water);

V6 - Holy water;

V7 - Peat + water;

V8 - Peat + holy water.

The seeds from each repetition were placed for germination on the surface of a layer of filter paper (S), which at wetting (in equal quantities per variant) was about 2 mm thick, in Petri dishes, in the V7 and V8 variants the seeds were placed for germination on peat substrate.

Figure 5. Aspects during the experiment

Figure 6. Aspects during the experiment

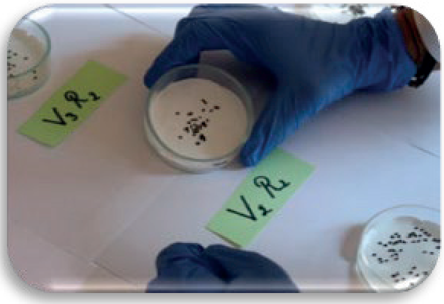

Figure7. Aspects during the experiment

In order to meet the requirements of germination, the moisture content of the germination substrate must be sufficient at all times, but not in excess. The studied seeds were moistened with equal amounts of liquid, avoiding variation between results.

The seed temperature was 25°C.

The results were statistically assured.

RESULTS AND DISCUSSIONS

SMARALD BASIL GERMINATION TEST							
NR. CRT.	Variant				t	P%	MEANING
1	V1 Mt.	20,33	100,00	0,00	0,00	92,00	
2	V2	23,67	116,39	3,33	2,51	2,50	*
3	V3	21,00	103,28	0,67	0,50	62,40	
4	V4	22,00	108,20	1,67	1,25	24,80	
5	V5	25,00	122,95	4,67	3,51	0,32	**
6	V6	26,00	127,87	5,67	4,26	<0,10	***
7	V7	16,00	78,69	-4,33	-3,26	92,00	
8	V8	19,67	96,72	-0,67	-0,50	92,00	
	x mediu	21,71					

Figure 8. Test values regarding the germination of 'Smarald' basil seeds

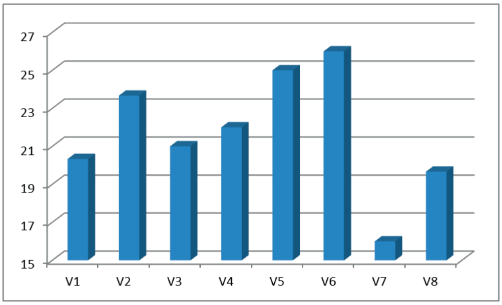

Figure 9. Graphic representation regarding the germination of 'Smarald' basil seeds

Following the research carried out in the Laboratory of Physiology, Agrochemistry and Ecological Cultures, from the Buzău Vegetable Research and Development Station, on the seeds of *Ocimum basilicum* var. *minimum* L.- The dwarf basil variety 'Smarald' was found that in the variant V6 - Sanctified water there were very significant differences, having the highest number of germs, compared to the variant V1 - Water (control). Distinctly significant differences were found in variant V5 - Decoct (100 g plant/2000 ml water), compared to the control, and significant differences were found in variant V2 - Infusion (concentration 25 g plant/250 ml water), compared to control variant, the other study variants showing insignificant differences.

TEST GERMINAȚIE BUSUIOC SFÂNT							
NR. CRT.	Variant				t	P%	MEANING
1	V1 Mt.	42,67	100,00	0,00	0,00	92,00	
2	V2	38,67	90,63	-4,00	-2,07	92,00	
3	V3	41,00	96,09	-1,67	-0,86	92,00	
4	V4	45,00	105,47	2,33	1,21	24,80	
5	V5	45,00	105,47	2,33	1,21	24,80	
6	V6	42,67	100,00	0,00	0,00	92,00	
7	V7	11,33	26,56	-31,33	-16,24	92,00	
8	V8	28,00	65,63	-14,67	-7,60	92,00	
	x mediu	36,79					

Figure 10. Test values regarding the germination of Holy basil seeds

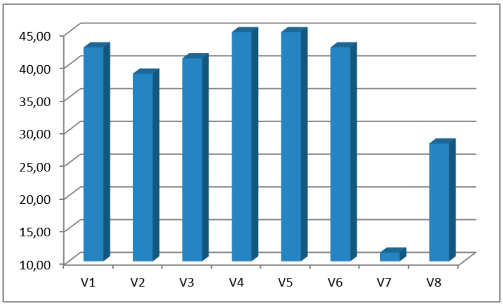

Figure 11. Graphic representation regarding the germination of Holy basil seeds

Following the research carried out in the Laboratory of Physiology, Agrochemistry and Ecological Cultures, from the Buzău Vegetable Research and Development Station, on the seeds of *Ocimum sanctum* Linn. - Basil, it was found that in variants V4 - Infusion (concentration 25 g plant/500 ml water) and V5 - Decoction (100 g plant/2000 ml water) were the highest values for the number of germs, compared to the variant V1 - Water (control), but the differences were not significant. The other variants in the studio showed lower values than the control.

TEST GERMINAȚIE BUSUIOC SERAFIM							
NR. CRT.	Varianta				t	P%	SEMNIFICATIA
1	V1 Mt.	20,67	100,00	0,00	0,00	92,00	
2	V2	20,00	96,77	-0,67	-2,42	92,00	
3	V3	20,67	100,00	0,00	0,00	92,00	
4	V4	22,00	106,45	1,33	4,84	<10	***
5	V5	21,33	103,23	0,67	2,42	3,00	*
6	V6	25,33	122,58	4,67	16,94	<10	***
7	V7	22,33	108,06	1,67	6,05	<10	***
8	V8	20,67	100,00	0,00	0,00	92,00	
	x mediu	21,63					

Figure 12. Test values regarding the germination of 'Serafim' basil seeds

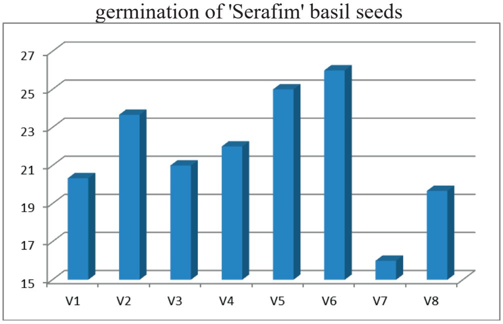

Figure 13. Graphic representation regarding the germination of 'Serafim' basil seeds

Following the research carried out in the Laboratory of Physiology, Agrochemistry and Ecological Cultures, from the Buzău Vegetable Research and Development Station, on the seeds of *Ocimum basilicum* L. var. *violaceum hort*. Purple basil 'Seraphim' was found that in the variant V6 - Sanctified water there were very significant differences, having the highest number of germs, compared to the variant V1 - Water (control). Very significant differences were also recorded in the variants V4 - Infusion (concentration 25 g plant/500 ml water) and V7 - Peat + water compared to the control. In the variant V5 - Decoct (100 g plant/2000 ml water) significant differences were found compared to the variant V1 - Water (control), the other study variants showing insignificant differences.

CONCLUSIONS

The following conclusions can be drawn from the research:

- For the establishment of innovative cultivation technologies for *Ocimum basilicum* we must have the optimal method of germination.
- From the tests performed in the Laboratory of Physiology, Agrochemistry and Ecological Cultures, from the Vegetable Research and Development Station Buzău, regarding the germination of the seeds of the three basil varieties, it was found that the 'Smarald' basil seeds germinated in large numbers in the experimental variant V6, compared to the control variant. The same was found in the test carried out with 'Serafim' basil seeds. Holy basil seeds germinated well in the experimental varieties V4 and V5, but with a value very close to them was the study variety V6.

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ASPECTS REGARDING THE ANATOMY OF THE STEM AND LIFETIME AS CUT FLOWERS OF SOME DAHLIAS CULTIVARS

Elena Cristina ȚURLEA (CIOBANU), Mihaela GEORGESCU, Monica Luminița BADEA, Mihai Ioan CIOBANU, Sorina PETRA, Florin TOMA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: ciobanuelenacristina@yahoo.com

Abstract

Plants of the Dahlia genus are particularly valued for their wide variety of shapes and colors both as cultivated plants and as cut flowers thus increasing the interest for the introduction in the culture of cultivars with great decorative value and great resistance as cut flowers. The work presents anatomical aspects, but also particularities regarding the life span of cut flowers in preservation solutions for the cultivars 'Topmix Red', 'Hy Pimento', 'Babylon Red', 'Marble Ball' and 'Thomas Edison'. The objective of this study was to analyze dahlia varieties both from the anatomical point of view and the preservation of cut flowers in vases using different preservation solutions to establish a correlation between the diameter of xylem vessels and cut flower life. The diameter of the xylem vessels does not seem to influence the life span of the cut flowers, regardless of the solution used in the conditions of this experiment. The Quick dip and eZDose conservation solution increased the lifespan of the varieties studied compared to the other conservation solutions used.

Key words: *cross-sections, cut flowers, dahlia, flower longevity, xylem.*

INTRODUCTION

Dahlias are native to Mexico. Here, the first dahlia flowers appeared, the first species that constituted inexhaustible sources for the emergence of new varieties, some of which are more interesting due to the flowers' composition, size, or color (Șelaru & Mucescu, 1976). Dahlia has a fistulous stem, 30-200 cm high. The main stems form several branches that give an appearance garnished with shoots, leaves, and flowers (Pârvu, 2003). Dahlias have leaves colored in intense green, bright, and pinnately lobed, with sharp and dented lobes on the edge (Toma, 2009). Dahlia flowers are grouped in calathides (Berar et al., 2006), and they bloom profusely from July to late autumn (Băla, 2007). The role of the flowers is particularly important in human life, fulfilling several essential functions: aesthetic, social, sanitary, and socio-economic (Toma, 2003). The richness, splendor, and beauty of the landscapes that can be created in parks and gardens by cultivating perennial flowers, constitute an enchantment (Rovența, 1968). Being used for aesthetic, artistic (sources of inspiration for painters, poets, and composers), and sanitary purposes (they maintain the balance in the composition of the air, retain dust from the atmosphere, and maintain a higher atmospheric humidity), flowers definitely influence the physical and mental state of man. Dahlia is a flower that can be grown in any kind of garden, alone or combined with other annuals or perennials. The way Dahlia is used to decorating the private garden depends on the available space and the personal ideas and wishes of each dahlia lover, but to make something interesting and special it is advisable for the private grower to ask for the advice of a specialist or visit different public gardens where inspiration can be found (Cantor et al., 2012). The aesthetic importance of flowering plants grows and becomes increasingly complex under the conditions of the contemporary era (Şelaru, 2002). This cut flower has very particular characteristics, becoming one of the most beautiful flowers abroad; furthermore, it is the single plant that has the largest number of cultivars of all plant species and more than 50,000 have been registered with the Royal Horticultural Society of England (Bye & Linares, 2008).

Cut flowers account for about half of the market for horticultural products, with

developed countries consuming more than 90%. Many consuming countries do not have ideal climatic conditions for the production of cut flowers so the flowers grow in protected environments, therefore production prices increase (Ovando et al., 2006). Cut dahlia flowers (Dahlia Cav.) have long been popular with consumers, and their relatively short longevity after harvest means growers and retailers are looking for ways to extend the life of potted flowers (Bergman et al., 2019).
The objective of this study was to analyze dahlia varieties both from the anatomical point of view and the preservation of cut flowers in vases using different preservation solutions to establish a correlation between the diameter of xylem vessels and cut flower life using different preservation solutions.

MATERIALS AND METHODS

The biological material was represented by dahlia plants grown in the "I. Todor" Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine Bucharest. The cultivars studied were 'Topmix Red', 'Hy Pimento', 'Babylon Red', 'Marble Ball' and 'Thomas Edison' (Figure 1).

Figure 1. Dahlia culture in the "I. Todor" Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine Bucharest
(Source: original)

The studies were carried out during the year 2022. Anatomical observations and measurements were made on cross-sections from the apical and basal areas of the floral stem. The sections were clarified with chloral hydrate and stained with carmine-alum and iodine green, according to the classical method (Morlova et al., 1966). The following anatomical needle traits were measured: E = Epidermis; C = Cuticle; H = Hypodermis; M = Mesophyll; Rc = Resin canals; En = Endodermis; Tt = Transfusion tissue; Vb = Vascular bundles; X = Xylem; Ph = Phloem. Observations, measurements, and photographs were taken with the LEICA DM 1000 LED optical microscope, LEICA DFC 295 video camera, and LEICA S 8 APO stereomicroscope, using 4x, 10x lenses, existing in the endowment of the Research Center for Studies of Food Quality and Agricultural Products (USAMV of Bucharest).
As far as highlighting the lifespan of dahlia cut flowers is concerned, tap water was used for the control sample, and the preservation solutions taken in the research were represented by Quick Dip and eZDose, Floralife Express No Cut, and Finishing Touch (Figure 3).
All stems from the three sources were kept in water from the time of cutting until arrival at the laboratory (30 minutes) and 3 repetitions were performed within the experiment. Stems were cut to 30 cm and all leaves were removed before being placed in the treatments used. Harvesting took place in the morning, the inflorescences were fully opened. All solutions were made with tap water and the stems were placed in 250 ml vessels and the storage temperature was 22°C. The shelf life of the cut flower in the pot is over when half of the flower has faded and wilted (Figure 2).

Figure 2. Wilted and discolored inflorescence (Dahlia 'Thomas Edison') (Source: original)

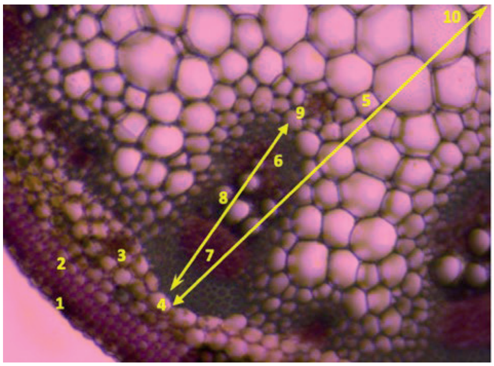
Figure 3. Studied cultivars: 'Topmix Red', 'Hy Pimento', 'Babylon Red', 'Marble Ball' and 'Thomas Edison' (Source: original)

RESULTS AND DISCUSSIONS

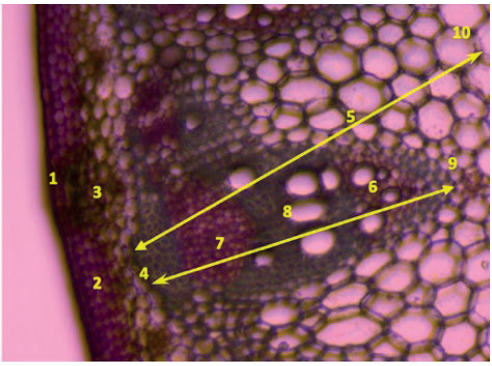
Anatomical observation

The epidermis has a single row of cells, tangentially elongated; the outer cell walls are covered by a thin layer of cuticle. Collenchyma has four layers of an angular type beneath the epidermis representing the first cortex zone (or hypodermis). Chlorenchyma: the second cortex zone is a parenchyma with chloroplasts, consisting of 2-3 cell layers. Endodermis has the inner zone of the cortex is visible, and it is composed of a single layer of tangentially elongated cells. In the Central Cylinder, vascular bundles are arranged on a single circle, larger bundles alternating with smaller ones. A vascular bundle is of collateral type, edged by a sclerenchyma tissue; the vascular cambium has finished the annual cell production, and there are no more visible traces of cambium activity between the phloem and xylem zone. At the end of some vascular bundles, secretory channels can be seen towards the pith area. They are surrounded by collector cells. The pith is represented by a parenchyma with lacunar collenchyma from place to place (Figure 4).

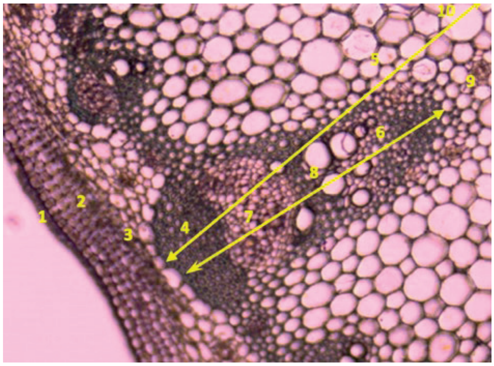
'Marble Ball'



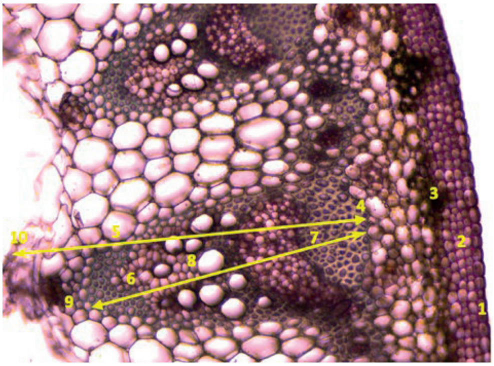
'Hy Pimento'



‘Topmix Red’



‘Babylon Red’



‘Thomas Edison’

Figure 4. Cross section through the stem from 5 cultivars of Dahlia: ‘Marble Ball’, ‘Hy Pimento’, ‘Topmix Red’, ‘Babylon Red’ and ‘Thomas Edison’
Legend: epidermis (1), collenchyma (2), chlorenchyma (3), endodermis (4), central cylinder (5), vascular bundle (6), phloem (7), xylem (8), secretory channels (9), the pith (10)
(Source: original)

Table 1. Diameter of xylem vessels and vase life to Dahlia

Cultivar	Fresh-keeping agent	Vase life (days)	Diameter of xylem vessels (μm)
'Topmix Red'	Tap water	3.50	23.08
	Quick dip + eZDose	5.00	23.08
	Floralife Express No Cut	4.33	23.08
	Finishing Touch	4.00	23.08
'Hy Pimento'	Tap water	5.50	23.46
	Quick dip + eZDose	6.50	23.46
	Floralife Express No Cut	6.33	23.46
	Finishing Touch	5.33	23.46
'Babylon Red'	Tap water	5.00	23.96
	Quick dip + eZDose	6.50	23.96
	Floralife Express No Cut	5.99	23.96
	Finishing Touch	5.50	23.96
‘Marble Ball’	Tap water	3.50	21.99
	Quick dip + eZDose	6.33	21.99
	Floralife Express No Cut	5.66	21.99
	Finishing Touch	5.00	21.99
‘Thomas Edison’	Tap water	4.67	24.61
	Quick dip + eZDose	5.50	24.61
	Floralife Express No Cut	4.99	24.61
	Finishing Touch	4.83	24.61

A very weak correlation between the diameter of xylem vessels (μm) and vase life (days) was recorded (R^2 = 0.043, y = - 0.1311 x + 24.812, where y = diameter of xylem vessels and x = vase life). The diameter of the xylem vessels does not seem to influence the life span of the cut flowers, regardless of the solution used in the conditions of this experiment (Table 1).

Observations on the lifespan of dahlia cut flowers

Analyzing Table 2, it is observed that there are statistically guaranteed differences between the 4 conservation solutions on the 5 Dahlia cultivars. The shortest storage period of 3.5 days was recorded for the cultivars 'Topmix Red' and 'Marble Ball' in the tap water solution (control sample), and the longest storage period of 6.5 days was recorded for the cultivars 'Hy

Pimento' and 'Babylon Red' in the Quick dip and eZDose solution.

Variants V_6 ('Hy Pimento'/Quick dip and eZDose) and V_{10} ('Babylon Red'/Quick dip and eZDose) with a count of 6.5 days in the preservation solution are distinctly statistically significant compared to the other variants. Variants $_7$ ('Hy Pimento'/Floralife Express No Cut) and V_{14} (‘Marble Ball’/Quick dip and eZDose) with a number of 6.33 days are statistically significant. The lowest value of 3.50 days specific to variants V_1 (‘Topmix Red’/Tap water) and V_{13} (‘Marble Ball’/Tap water) makes these variants highly significantly negative compared to the other 18 variants. Variant V_4 (‘Topmix Red’/Finishing Touch) is statistically significantly negative, and the variants V_2, V_3, V_5, V_8, V_9, V_{11}, V_{12}, V_{15}, V_{16}, V_{17}, V_{18}, V_{19}, V_{20} show insignificant differences.

Tabel 2. The influence of conservation solution on the taking of the decorative value of Dahlia flowers

Var. no.	Cultivar	Fresh-keeping agent	Vase life (days)	Relative vase life (%)	+/- days	Signification of the difference
1	‘Topmix Red’	Tap water	3.50	67.34	-1.70	000
2		Quick dip + eZDose	5.00	96.20	-0.20	-
3		Floralife Express No Cut	4.33	83.25	-0.87	-
4		Finishing Touch	4.00	76.90	-1.20	0
5	‘Hy Pimento’	Tap water	5.50	105.82	0.30	-
6		Quick dip + eZDose	6.50	125.06	1.30	**
7		Floralife Express No Cut	6.33	121.73	1.13	*
8		Finishing Touch	5.33	102.55	0.13	-
9	‘Babylon Red’	Tap water	5.00	96.20	-0.20	-
10		Quick dip + eZDose	6.50	125.06	1.30	**
11		Floralife Express No Cut	5.99	115.31	0.80	-
12		Finishing Touch	5.50	105.76	0.30	-
13	‘Marble Ball’	Tap water	3.50	67.34	-1.70	000
14		Quick dip + eZDose	6.33	121.85	1.14	*
15		Floralife Express No Cut	5.66	108.90	0.46	-
16		Finishing Touch	5.00	96.14	-0.20	-
17	‘Thomas Edison’	Tap water	4.67	89.79	-0.53	-
18		Quick dip + eZDose	5.50	105.82	0.30	-
19		Floralife Express No Cut	4.99	96.07	-0.20	-
20		Finishing Touch	4.83	92.93	-0.37	-
	Average		5.20	100	Martor	

LSD 5% = 0.95
LSD 1% = 1.27
LSD 0.1% = 1.67

Analyzing Figure 5, it can be seen that the lowest values specific to variant V1 ('Topmix Red') make this variant inferior to the other variants. The cultivar 'Hy Pimento' was best preserved in Quick dip and eZDose preservative solutions, followed by Floralife Express No Cut, Finishing Touch, and tap water preservatives, demonstrating that this cultivar was superior to the others in terms of resistance in the dish.

The preservation solution used influences the shelf life of cut flowers according to research by Ciobanu (2017), Dole et al. (2005), Lukaszewska (1986).

Regarding the maintenance of cut flowers, studies have shown that treatment with cytokinin or gibberellic acid can improve post-harvest quality and longevity. It was also shown that maintaining cut dahlia flowers in a solution containing both benzyladenine and gibberellic acid at 10-20 mg L^{-1} improved flower quality after 4 days in the vase and extended shelf life (Bergman et al., 2019).

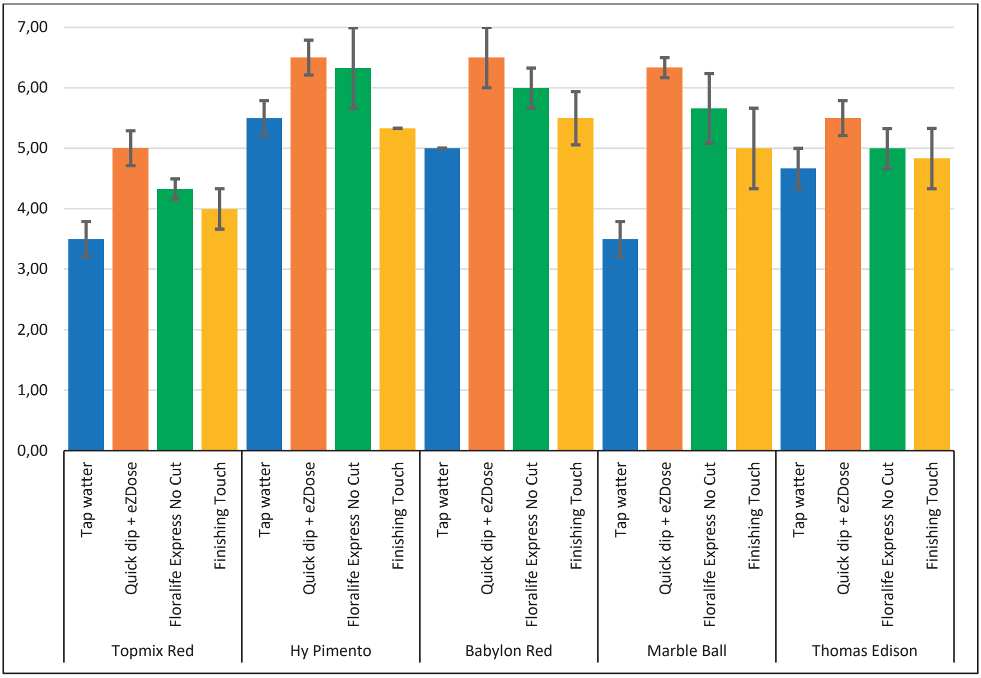


Figure 5. The lifetime (days) of five Dahlia cultivars in conservation solution (Tap water, Quick dip and eZDose, Floralife Express No Cut, Finishing Touch)

Regarding the improvement of the vase life of Dahlia cut flowers, a breeding research program using conventional cross-breeding techniques was carried out. Significant differences were found in terms of flower life in the vase, namely: nine cultivars had a long vase life (e.g. 'Syukuhai', 'Rinka' and 'Micchan'); eight had a normal vase life (e.g. 'Kamakura', 'Agitate' and 'Benifusya'); and seven had a short vase life (e.g. 'Gin-Ei', 'Port Light Pair Beauty' and 'Yumesuiren'). 22 cultivars were used as initial breeding materials, repeatedly crossed, and promising progeny with long vase life were selected for three generations. The results strongly suggest that 'Micchan' has genes related to long flower life in the vase and that the trait is heritable (Onozaki & Azuma, 2019).

The vase life of flowers is one of the most important traits for ornamental plants. The lifespan of cut flowers of dahlia (Dahlia variabilis) is very short, and genetic improvement of this trait is desirable (Onozaki & Azuma, 2019).

Studies by Shimizu-Yumoto and Ichimura K (2013), showed that dahlia flowers are sensitive to ethylene, but ethylene production by floral organs did not increase significantly during flower senescence. Silver thiosulfate complex did not extend the lifespan of cut flowers in the vase, while 1-methylcyclopropene extended the lifespan of the flowers. When 6-benzylaminopurine was applied either on bouquets by dipping or on flowers by spraying, the life of the dahlia was extended. In addition, 6-benzylaminopurine treatment with immersion prolonged the lifespan of the bouquets more than the 1-methylcyclopropene treatment.

CONCLUSIONS

The importance of dahlias is given by the decorative qualities of the inflorescences, being appreciated both as a cut flower and in floral arrangements, both alone and in association with other flowers. They stand out through a very varied range of colors and shapes, thus increasing the interest in the introduction into the culture of cultivars with great decorative value and great resistance as cut flowers.

The ‘Topmix Red’ cultivar is a dwarf cultivator that fits as a decorative garden plant and is not suitable as a plant in the vase because it has a very small life durate in the vase.

‘Hy Pimento’ and 'Babylon Red' cultivars have a longer life in the vase and are suitable for decorating the house.

Conservation solutions have extended the life in the vase of 'Hy Pimento' and 'Babylon Red' cultivars (6.5 days/Quick dip and eZDoe solution), followed by the ‘Marble Ball’ cultivar (6.33 days/Quick dip and eZDoe solution) and ‘Thomas Edison’ (5.5 days/ Quick dip and eZDoe solution).

The Quick dip and eZDose conservation solution increased the lifespan of the varieties studied compared to the other conservation solutions used.

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MISCELLANEOUS

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REVIEW OF THE LCA ELEMENTS APPLYING TO THE MICROALGAE LIPID EXTRACTION

Carmen Gabriela CONSTANTIN[1], Burçin ATILGAN TÜRKMEN[2], Aurora DOBRIN[1], Ion CERTAN[1]

[1]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania
[2]Department of Chemical Engineering, Bilecik Şeyh Edebali University, 11011, Bilecik, Turkey

Corresponding author email: certan.ion@qlab.usamv.ro

Abstract

Although significant studies of microalgae have been published, there is no clear information regarding the advantages, challenges, or feasibility of polyunsaturated fatty acids (PUFA) production in a sustainable large-scale process. Such information on the current state of PUFA extraction applied to feed and food is particularly important for researchers and stakeholders to identify and apply the most sustainable technology. Based on highly cited academic articles and other digital libraries of academic journals, this study aims to provide a comparison between different microalgae lipid extraction methods through LCA parameters evaluation. PUFA extraction from microalgae Nannochloropsis sp. used as feedstock, is evaluated using methods such as ultrasound, microwave, supercritical fluid extraction, and accelerated solvent extraction in a comprehensive review. Extraction yield, nature of the extraction solvent, energy type source and consumption, labour, and extraction time influenced the specific LCA parameters, quantified for global warming potential, ecotoxicity potential, fossil resource scarcity, and cumulative energy demand. It is possible to reduce production costs and environmental impact by selecting the appropriate method and optimizing these parameters.

Key words: *life cycle assessment, global warming potential, fossil resource scarcity, cumulative energy demand, sustainability.*

INTRODUCTION

Nowadays, in marine ecosystems factors such as overfishing and resource depletion are considered threats. Therefore, there is a need to find and develop such methodologies to identify, quantify, and assess the main indicators related to marine biodiversity in order to maintain sustainable development.

Currently, fish and meat are the main sources of Polyunsaturated Fatty Acids (PUFAs), whereby meat causes high environmental impact, and fish catch cannot meet the demand of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Schade et al., 2020). PUFAs are components of cell membrane phospholipids serving as precursors for hormone-like inflammatory mediators. These long-chain acids are crucial for the health of humans and most animals, because they do not have the ability to produce them, so it is important that they are supplemented in the diet. This is why the demand for EPA and DHA has significantly increased (Sá et al., 2020; Togarcheti et al., 2021). It seems like the long chain PUFA are involved in regulation of cell metabolism at the nuclear level being related with the progression of chronic diseases. Scarcity in saturated acids is associated with the blood lipid profile (Shi et al., 2018).

However, the long-chain n-3 PUFAs, EPA, and DHA, are scarce nutrients in a global context. Therefore, a valuable renewable source of PUFAs may be represented by microalgae. Nannochloropsis sp. microalgae are easily grown at upscale conditions and can be used to synthesize high-value compounds for the pharmaceutical, cosmetic, and nutraceutical industries (Gaber et al., 2021; Ferreira et al., 2013). Due to the of the cell's structural properties, the identification of the proper solvent, and extraction technology for its components such as lipids remains the main challenge (Ferreira et al., 2013). It is critical to increase lipid productivity in order to improve the process's economic competitiveness. This can be achieved by reducing the high energy requirements associated with water management and lipid extraction from the biomass. Producing and quantifying fatty acids is a laborious and time-consuming process that includes several steps such as extraction, fractionation, methylation, and quantification. Furthermore, the process is not environmentally friendly because it uses various organic solvents in this step. It is vital to look into alternate lipid extraction techniques in order to make the process more environmentally friendly. These methods should be cost-effective and requiring less time and energy while ensuring high yields of fatty acids. This reduces the environmental impact of the process and makes it more sustainable (Sá et al., 2020).

In this context, a comprehensive accounting of the environmental sustainability assessment is required. Life Cycle Assessment (LCA) is an important tool for determining the most environmentally friendly process (Togarcheti et al., 2021; Barahmand et al., 2022).

Although the LCA is highly valued, the number of LCAs on PUFA products (Van Boxtel et al., 2015) produced by *Nannochloropsis* sp. is scarce, most of the studies being related to biomass production and biofuels obtaining (González-Delgado and Kafarov, 2011). Throughout time, the LCA and sustainability assessment was applied for the entire algal biodiesel processes. (ref) The LCA of algal biodiesel production process provided a quantitative measure for its sustainability. Even though the published LCA studies of algae biodiesel processes are reviewed, demonstrates that there are few comprehensive studies that cover the complete process. Therefore, the outcomes can be inconclusive. The variability of algal species, reactor type and conditions, and other factors influence the LCA outcomes. Also, on LCAs applied to the algae biodiesel process, a lack of systematic influence on the outcomes.

In recent decades, scientists have been concerned with balancing the costs of their findings with their long-term viability in order to develop renewable-energy-based products. Therefore, clear and standardized frameworks are necessary for the economic sustainability. The techno-economic analysis (TEA) and the life cycle cost analysis (LCCA) are two widespread methodologies to calculate the economic indicators. According to Giacomella (2021), the TEA's methodological steps may be related to the following steps: (a) technology readiness levels (TRL), (b) elements and boundaries, (c) market conditions, costs, and feasibility, (d) profitability, (f) risks and uncertainty, and (g) recommendations. Therefore, TEA's methodological steps comprise (1) problem definition and objectives, (2) cost analysis, (3) discounting future cash flows and economic evaluation, (4) considering risks and uncertainties, and (5) comparing the alternatives and possibilities.

Although the use of TEA is increasing, it is very difficult to define what TEA constitutes. However, researchers have defined the methodology, meaning that there are three key questions related to the mechanism and the profit of the technology, and whether the technology is desirable.

Despite their widespread acceptance, guidelines and comprehensive documentation on their features are limited (Barahmand et al., 2022). Therefore, based on the published scientific articles the aim of this study is to identify the main LCA system insights into the challenges of PUFA. The data will be adapted and applied to extraction techniques with Nannochloropsis sp. serving as a matrix.

LIFE CYCLE ASSESSMENT (LCA)

LCA is a tool used in environmental management to determine the material, energy, and waste flows and their potential impacts on the environment over the course of a process, product, or service. The evaluation covers the entire life cycle, including raw material extraction and processing, production, transportation, and distribution, use, re-use, maintenance, recycling, and final disposal (Muralikrishna & Manickam, 2017).

The International Organization for Standardization (ISO) established the ISO 14040 and ISO 14044 standards (ISO, 2006a, 2006b), which define the LCA methodology. As shown in Figure 1, LCA is composed of four interconnected steps based on this set of standards. These include impact assessment, inventory analysis, goal and scope definition, and result interpretation.

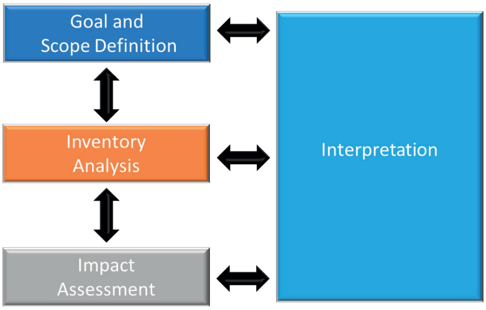

Figure 1. Life cycle assessment framework

The product system is defined in terms of the study's system boundaries and a functional unit during the goal and scope defining stage. For the purpose of comparing alternative products or services, the functional unit is crucial. The life cycle inventory (LCI) analysis stage is used to estimate the amount of resources consumed, waste produced, emissions, and other factors associated with each stage of a product's life cycle. The flows of energy and materials between stages of a life cycle are modelled. For each functional unit, the overall models offer mass and energy balances for the product system, including all of its inputs and outputs into the environment (Azapagic, 2010; Pennington & Rydberg, 2005). The potential environmental impacts resulting from the elementary flows (environmental resources and releases) obtained in the LCI are evaluated in the impact analysis (LCIA) stage. First, the environmental impact categories relevant to the study are chosen and defined, and the environmental impacts are calculated by multiplying the inventory items by the relevant coefficients (Guinée et al., 2011). Environmental impacts including emissions, energy, carbon, water, toxicity, ozone depletion, eutrophication, acidification and resource depletion are impact categories commonly assessed in LCA studies. Different impact assessment methods such as TRACI, Ecoindicator, ReCiPe and CML methods. Selected environmental impact categories are presented as an example in Figure 2. The final step involves a thorough analysis of the data to identify the major environmental impact categories and impact hotspots. It is then possible to use these to suggest ways to improve (Azapagic, 2010).

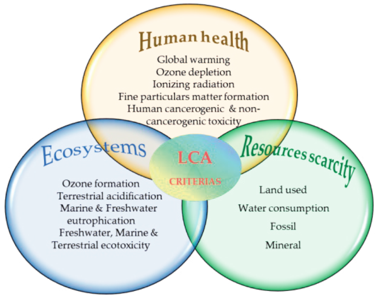

Figure 2. Environmental impact categories based on ReCiPe impact assessment method

LIFE CYCLE ASSESMENT OF LIPID EXTRACTION FROM MICROALGAE

This paper provides a thorough comparison of the environmental sustainability of various microalgae lipid extraction methods. For these purposes, studies on life cycle assessment found in the literature have been reviewed. Using "LCA parameters on microalgae PUFA extraction" as searching words, on Science Direct were only 166 results from 2005. The highest number of 37 articles related to the key words were found in 2022 (Figure 3). When the studies in the literature are examined, it is discovered that they differ in terms of scope, purpose, target, and techniques used. This section of the paper reviews and analyses LCA studies using the LCA methodology.

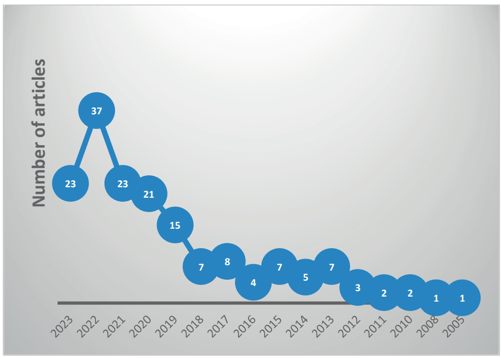

Figure 3. Number of articles in Science direct platform related with LCA parameters and microalgae PUFA extraction

1. *Goal and scope definition*

The aim of the study, the product or process system in terms of the system boundaries, and a functional unit are all described in the goal and scope definition of an LCA. The reviewed studies have different goal and scope The reviewed LCA studies' functional units vary based on the goals and parameters of the research.

Bartek et al. (2021) aims to investigate and compare the environmental impact of docosahexaenoic acid (DHA) produced from algae with substrate derived from dark fermentation (DF) using food waste to that of DHA produced from Peruvian anchovy oil. The functional unit used was selected as 1 kg of lipids (neutral and free fatty acid). At this point, three lipid extraction scenarios were described: CHCl3-MeOH, CO_2 expanded methanol, and non-expanded methanol.

Comparing the environmental effects of various Nannochloropsis sp. and P.tricornutum cultivation scenarios with aquaculture and capture fish production as conventional sources of EPA and DHA is the goal of the another study (Schade et al., 2020). In study, as the functional units was kg DM (dry mass) for microalgae and 1 kg of fresh fish fillet, for protein 50 g and 500 mg EPA+DHA, corresponding with daily intake recommendations per person (World Health Organization).

In their research, Medeiros et al., (2022) quantify the energy demand, economic sustainability, and global warming potential of microalgae biomass production. The product system consists of carbon dioxide-injected open raceway ponds for microalgae cultivation, followed by settling, filtration, and centrifugation for harvesting. A kilogram of microalgae biomass production in total solids with 80% moisture has been used in the evaluated scenarios.

Paramita (2012) in his thesis analysed the modelling of extraction processes (hexane extractions and supercritical fluid extraction CO_2. Also, in this case the basis for inventory 1 kg of oil in the algae biomass was used to analyse efficiency, effectiveness and environmental impact.

Shi et al. (2018) investigated the environmental impacts of different harvesting and extraction technologies. The gate-to-gate system boundaries are chosen. In the study, two different functional units were chosen. The functional unit for harvesting technologies was determined as 1 kg of dry algae biomass harvested, while the functional unit for extraction technologies was determined as 1 MJ lipid oil output.

Also, Papadaki et al. (2016) aims to evaluate the environmental sustainability linked to various extraction techniques for astaxanthin recovery. Using microwaves and ultrasonics, a comparative study was conducted between traditional solvent extraction and novel green extraction techniques to recover bioactive compounds, particularly astaxanthin, from Haematococcus pluvialis microalgae. A life cycle assessment, encompassing the cultivation, harvesting, and extraction treatment up to the production of extracts rich in astaxanthin, was carried out. The functional unit was defined as 1 kg equivalent (eq.) of astaxanthin recovered from *H. pluvialis* to be used as an additive in cosmetics and nutraceutical applications.

Through process simulation, Lopes et al., (2023) developed the conceptual design of a microalgae production plant, as well as its harvesting, dewatering, cell disruption, and aqueous fraction processes. The functional unit was defined as 1 kg of ash-free dry-weight biomass produced and processed in the system.

2. *Life cycle inventory*

According to the literature, there are several aspects that should be considered for the inventory analysis stage.

LCI applied in microalgae production:

- LCA system boundaries of upscaled total fatty acid (TFA) production (Gaber et al., 2022; Schade et al., 2020);
- Scheme of energy inputs (Ferreira et al., 2013);
- Flowchart of microalgae production and processing into various fractions of interest (Lopes et al., 2023; Zi Hao et al., 2023);
- Influence of the life cycle stages on the environment parameters (Medeiros et al., 2022).

According to Medeiros et al. (2022), the following variables were used in the analysis for cultivation and harvesting:

- Input: occupied area, water, saline, infrastructure (concrete, polypropylene, excavation, steel, PVC, pipe, cast iron, CO_2), synthetic fertilizer), transport of fertilizer, truck, electricity, labor.

- Output: water, air, CO_2 loss, air, water, blowdown effluent, biomass-loss.

For energy demand, carbon footprint and financial cost contributions of microalgae biomass production the following contribution groups were analyzed: cumulative energy demand (CED), global warming potential (GWP) and capital and operational cost Medeiros et al. (2022).

Lopes et al. (2023), in their work, created a process model that can simulate an industrial plant to estimate mass and energy balances, optimize scheduling, and calculate production costs for a large-scale plant. They also combined TEA and LCA, like Medeiros et al. (2022). In their study, four scenarios and three microalgae strains (*Nannochloropsis* sp., *Dunaliella* sp. and *Spirulina* sp.) were considered. In the scenarios, they analyzed the following parameters: water recirculation, no water recirculation, industrial gaseous CO_2 as a carbon source and flue gas as a carbon source. Each scenario was applied to each microalgae.

Two scenarios are chosen for analysis in the study conducted by Bartek et al. (2021): the conventional fish scenario, in which DHA is obtained from Peruvian anchovy, and the conceptual algae scenario, in which DHA is produced from C. cohnii microalgae using volatile fatty acids (VFA) derived from DF with food waste. The energy used for building construction, processing, end-of-life, and transportation of necessary inputs is included in the system boundary calculation.

Ferreira et al. (2013) analyzed energy inputs and CO_2 emissions for the microalgae culture and downstream processing comparing two methods of oil extraction: supercritical fluid extraction (SFE) and Soxhlet extraction (SE) for bioH2 production. They used as inputs: nutrients (N, K, P, Fe, Na, B, Mn, Zn, Co), deionized water, light intensity, centrifugation, drying, cutting mill and ball mill, fermentation, sterilization, drying and evaporation.

Gaber et al. (2022) considered for LCA analysis two systems upstream (algae cultivation and harvest with centrifuges) and downstream (cell disruption using homogenization, the extraction of TFA, biodiesel production, and solvent vaporization) to obtain the final TFA product. In LCI four extraction methods were used: ultrasound, microwave, supercritical fluid extraction, and accelerated solvent extraction, being very important to identify the wide range of parameters and to perform a comprehensive analysis. Therefore, the following parameters were analyzed:

- Pilot reactor and 20-ha upscaled plants:
- Electricity consumption (skid electricity, harvesting electricity, seawater recycling, TFA extraction via solvent and 3-phase separator, cell homogenizer, solvent vaporization);
- Demand of nutrients (sodium nitrate, sodium phosphate, ferric chloride).
- CO_2, cleaning materials and solvents as operational materials;
- Sodium hypochlorite, hydrogen chloride, TFA extraction via solvent and 3-phase separator, cell homogenizer oil, land use),
- Water consumption (cooling water for chiller, cleaning water, cell homogenizer water).

LCI applied in fish feed production and fish growing consider three main costs:

- Energy for feed production, and fish growing in Recirculating Aquaculture System (RAS) (Schade et al., 2020);
- Investment in infrastructure for fish growing;
- Water consumption in RAS (Schade et al., 2020).

3. *Impact assessment*

For the environmental impact study, Restuccia et al. (2022) used SimaPro software, midpoint ReCiPe 2016 (H). This methodology was chosen and preferred over other calculation methods such as ILCD 2011, CML 2001 or TRACI because of the availability of eighteen impact categories (compared to 16 from ILCD 2011 Midpoint, 15 from IMPACT 2002+, 11 from CML-IA Baseline, and 9 from TRACI).

For impact assessment, Gaber et al. (2022) in their study mentioned OpenLCA version 1.7 software with unit processes selected from the LCA database Ecoinvent 3.4 in accordance to ISO 14040/44. The same ISO was also used by Ferreira et al. (2013), Papadaki et al. (2016), Lopes et al. (2023) and ILCD Midpoint 2011 impact assessment method, developed and promoted by the Joint Research Council (JRC) of European Commission, being a very useful tool in order to evaluate fatty acids extraction from microalgae. It included the following aspects: climate change, particulate matter formation, freshwater eutrophication, mineral resource depletion, water resource depletion, and land use. Medeiros et al. (2022) used openLCA v.1.11.0 with the Ecoinvent v.3.6 and TEA method was used for the economic performance. LCA and TEA were integrated for the same goal, scope and foreground inventory.

Into the description of result aggregation were presented the following categories: processes (electricity flows, infrastructure flows, nutrient flows, tap water flows), LCA phases (cultivation, hydrothermal liquefaction and downstream parameters). Togarcheti et al. conducted a study in 2021 that used inventory data from the literature to predict the primary energy demand and environmental impacts associated with unit operations for the production of EPA+DHA from microalgae, and the characterisation methods used were ReCiPe 2016 Midpoint (H) v1.06 and the single-issue method of CED v1.11. In the environmental impact was assessed the following parameters: abiotic depletion potential (ADP), eutrophication potential (EP), GWP and acidification potential (AP). Schade et al., (2020) compared different cultivation scenarios of *Nannochloropsis* sp. and *P. tricornutum* with the production of aquaculture and capture fish as traditional sources of EPA and DHA, using Ecoinvent database v3.4. The following impact categories are used in the analysis: ADP, GWP, Ozone Depletion Potential (ODP), Human Toxicity Potential (HTP), AP, EP, Photochemical Oxidation Potential (POCP), Terrestrial Ecotoxicity Potential (TETP), Marine Aquatic Ecotoxicity Potential (MAETP) and Fresh Water Aquatic Ecotoxicity Potential (FAETP).

Lopes et al., (2023) used SimaPro v9.4.0.1 with the Ecoinvent database v.3.8 and by applying the environmental footprint impact assessment method (EF 3.0). A Monte Carlo simulation was also performed to estimate the possible outcomes of an uncertainty. The environmental impact distribution (%) per sub-system and for each scenario, as well as the environmental impacts for each strain for the scenarios under consideration, were analysed. The following impact categories has been analysed: climate change; ozone depletion; ionizing radiation; photochemical ozone formation; particulate matter; human toxicity, non-cancer; human toxicity cancer; acidification; freshwater eutrophication; marine eutrophication; terrestrial eutrophication, freshwater ecotoxicity; land use; water use; fossil resource use.

Papadaki et al., (2016) used LCA impact assessment method using Simapro 7.1 software and the CML2 baseline 2000 method, for the potential environmental damage of airborne, liquid and solid emissions by means of appropriate equivalence factors to selected reference compounds.

For hexane extraction has been used as follows by Paramita, 2012: from technosphere (oil mill, tap water, hexane, phosphoric acid, electricity and heat) and to atmosphere (hexane). The following procedures have been used for supercritical fluid extraction with CO_2: from the technosphere (CO_2, electricity, compression, electricity, cooling water, tap water, and to the atmosphere (CO_2) (Figure 4 a, b).

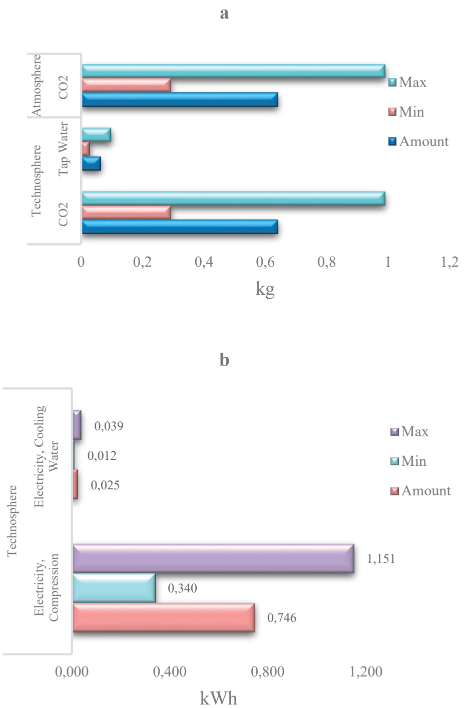

Figure 4. Inventories for supercritical fluid extraction CO_2 ($SCCO_2$) (Paramita, 2012)

Other parameters that were compered in LCA analysis by Paramita et al. (2012): productivities and lipid content of microalgae, nutrient and CO_2 inputs to the growth system, CO_2 compression, daily water need for the whole facility, energy need for water transport and treatment, harvesting via in-pond sedimentation, producing 1 time harvest worth of biomass, harvesting, dewatering, and drying processes, hydrothermal liquefaction, transesterification following hexane or $SCCO_2$ extraction to produce 1 kg of biodiesel, transesterification following hydrothermal liquefaction to produce 1 kg of biodiesel, hydrotreating following hydrothermal liquefaction to process 1 kg of algal oil, comparison non-renewable energy input in using green algae and diatom, hydrotreating. Shi et al. (2018) analysed the GHG emissions and converted to CO_2 eq. using the Intergovernmental Panel on Climate Change (IPCC) 2013 GWP 100a method in the SimaPro software. CO_2, CH_4, and N_2O GWP values were included, with 100-year GWP values of 1, 28, and 265, respectively. Inventories of various inputs has been calculated, including the energy inputs (electricity, heat, and steam), chemicals and solvents, as well as other material inputs. The study conducted seven gate-to-gate LCAs unit technologies and evaluated 14 scenarios of incorporating different harvesting and extraction technology combinations into the full algae life cycle. Harvesting technologies considered included Chitosan flocculation, electrolytic coagulation, membrane harvesting, and acoustic harvesting; while extraction technologies considered included wet separation/fractionation (AlgaFracTM) and acoustic extraction (Figure 5).

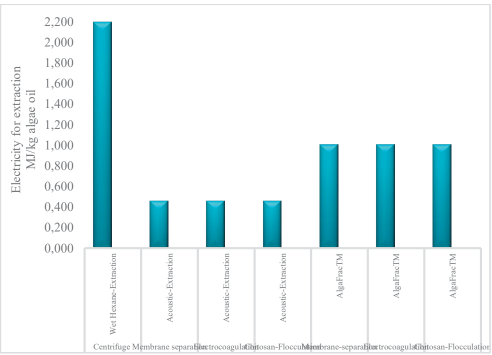

Figure 5. Extraction technologies: Wet Separation/ Fractionation (AlgaFracTM) and Acoustic Extraction, after Shi et al., 2018

RESULTS AND DISCUSSIONS

In the resource efficiency scenario results, Gaber et al. (2022) presented that hydrothermal liquefaction using aqueous phase during cultivation can reduce energy demand, nutrient input, revealed a decreased by 0.8 kg CO_2 from the Baseline Scenario. The authors also compared TFA extracted from microalgae with those from soybean. 1 kg of soybean oil requires far more land than 1 kg of algal TFA, in the context of microalgae cultivation with saltwater instead of tap water. As a result, since soybean production requires inputs like tap water for irrigation, a negative impact from soybean oil production rather than algal TFA was anticipated in the case of water depletion. Medeiros et al. (2022) used N and P from residual sources and energy from photovoltaic systems for microalgae biomass production. The results showed a decrease in energy (61%), carbon footprint (84%), and financial cost (37%).

Nutritional profile of microalgae and fish species per kg DM was also have been presented by Schade et al. (2020), microalgae biomass having higher content of fat and calories then alaska pollack, codfish and tilapia fish (Figures 6 and 7). Related to EPA content, *Nannochloropsis* sp. was highlighted having the highest content of all fish from both capture and aquaculture (Figure 8).

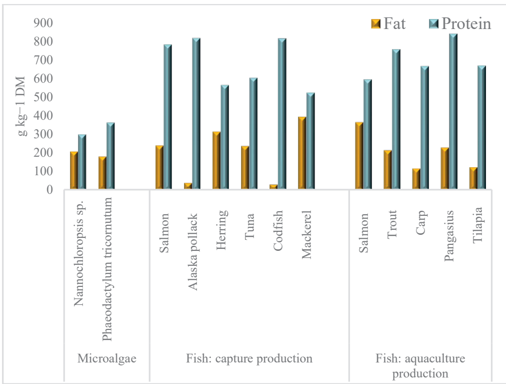

Figure 6. Fat and protein content of microalgae and fish species

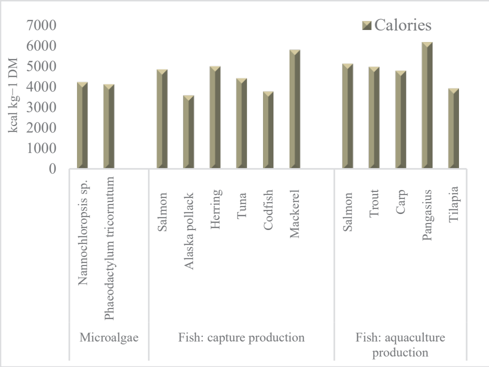

Figure 7. Calories content of microalgae and fish species

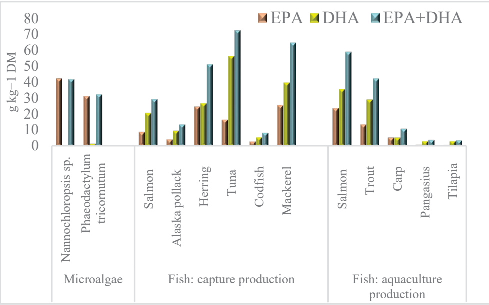

Figure 8. EPA and DHA content of microalgae and fish species

For aquaculture fish production, water and land used were taken into account, global warming was assessed as CO_2 eq. according to IPCC 2013 GWP 100(Directive 2014/24/EU, 2014). Acidification and eutrophication were recorded as SO_2 eq. and PO_4^- eq., respectively, based on CML-IA Baseline EU25 (Directive 2014/25/EU, 2014).

Schade et al. (2020) in their analysis showed that microalgae can be produced with lower environmental impacts than fish production.

Microalgae biomass cultivation that includes the burden of CO_2 production has similar or lower environmental impacts than aquaculture fish. Microalgae cultivation being sustainable in a temperate climate and are able to compete with fish as an alternative nutrient resource. Schade et al. (2020) for CO_2 scenarios the authors found that eutrophication potentials are similar to those of aquaculture fish production and slower emissions than with trout and pangasius.

Since farmed fish requires EPA+DHA as a supplement in its feed, a scenario was developed by supplementing EPA+DHA from microalgae and less from than caught fish. Has been demonstrated that the environmental impact of EPA+DHA production from farms was similar when fish oil from caught fish is replaced with oil from microalgae cultivated in the heterotrophic mode, proving that omega-3 fatty acid-producing in this way could be an alternative to conventional fish oil (Papadaki et al., 2016).

Lopes et al. (2023) developed a mathematical model which allowed the estimation of microalgal production/ processing and the associated environmental impacts and costs. Its conceptual design and validation were undertaken on the basis of real industrial-scale production data obtained using three different microalgae: *Nannochloropsis* sp., *Dunaliella* sp., and *Spirulina* sp. Regarding production costs, Scenario 1 (no water recirculation applied and the use of industrial gaseous CO_2) was revealed to be the most economic option, whereas Scenario 2 (the use of flue gas as a carbon source) the most expensive strain to produce/process having the lower productivity, which was compensated for on the biorefinery side by the extraction of carotene and glycerol.

The environmental impacts of cultivation and harvesting, drying and extraction stages were evaluated by Papadaki et al. (2016) and showed that *H. pluvialis* is a high rich source of astaxanthin which can be recovered sufficiently using not only organic solvent but also edible vegetable and essential oils. Microwave assisted extraction is considered also a rapid and overall eco-friendly technique suffering though from low yielding due to thermal degradation of carotenoids in long processing times. The maceration and the Soxhlet extraction techniques are highly time consuming, expensive and potentially hazardous due to the large number of solvents used, Soxhlet also exhibits low yielding due to thermal degradation and high energy demand (Figure 9 a, b).

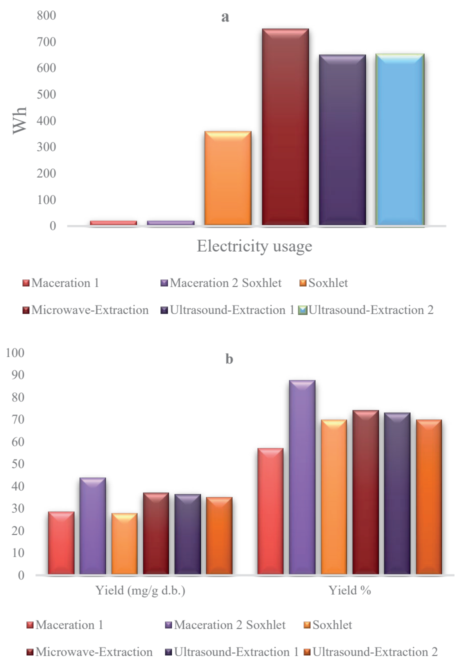

Figure 9. Extraction conditions, energy consumption and yield of *Haematococcus pluvialis* after Papadaki et al., 2016

The overall environmental impacts of the processes showed that abiotic depletion impact is minimized through the recycling of solvents and other materials though the cradle to gate process. The authors revealed that the selected solvents were assumed to be recycled and reused in a rate of 80 %, while vegetable oils were considered to be filtered and reused in a ratio of 50 % regarding scale up and industrial application.

In their study Shi et al. (2018) algae lipid content was assumed to be 25% for all scenarios, lipid density was assumed to be 864 kg per m^3, lower heating value of lipid was 10.5 kWh (37.8 MJ) per kg, and heating value of LEA was assumed to be 4.86 kWh (17.5 MJ) per dry kg.

From economically point of view, the most favorable *Nannochloropsis* sp. was oil, pigment and bioH_2 production via supercritical fluid extraction (SFE) then Soxhlet extraction (SE). From net energy balance and CO_2 emissions analysis, the biodiesel SE + bioH_2 presented better results, but in SFE it's possible to produce high-value oil and pigments with a clean technology free of toxic organic solvents (Ferreira et al., 2013).

Economic feasibility analysis

Shi et al. (2018) revealed that harvesting, drying and milling have an insignificant impact over total costs. The higher share of electricity consumption costs is caused by the relatively long (40 days) period of algae growth and by applied extraction type. It might be advantageous to reduce period of time that will lead to oil and pigment yields decrease, but the decision could be taken through permanent monitoring of the cellular accumulation of this compound. For Soxhlet extraction and pigment supercritical extraction (SFE) used for algal oil production. Although SFE is energetically more intensive, in monetary terms Soxhlet (660.56 €/kg) is almost twice expensive rather than the supercritical one (365.42 €/kg). Inputs like hexane affected the costs of Soxhlet process, making it more expensive. Processing a larger amount of biomass, close to an industrial scale, could lead to a decrease of energy consumption (and associated CO_2 emissions) and costs, as all of them were calculated based on a laboratory scale.

LCA analysis can be used together with TEA, both of them are critical for determining the environmental and economic risks and opportunities of a technology or product before industrial deployment.

For simple models, uncertain parameters of the method include lifetime, discount rate, fuel, consumables costs, and scaling exponents. Moderate systems include equipment sizes, costs, and escalation factors. Both simple and moderate parameters could be considered separated or included in complex models in addition with other parameters like scaling factors, detailed capital costs, operational costs (Barahmand et al., 2022).

CONCLUSIONS

The review brings new insides on LCA as tool for the sustainability PUFA extraction from microalgae.

The review highlights that LCA it is a useful analytical environmental management tool that can provide the environmental impacts from cradle to gate of microalgae production. Also, the analysis provides information related to PUFA extraction, but it has some limitation, and it can be recommended to be used together with techno-economic assessments (TEA).

For PUFA extraction, harvesting, drying and milling were found to be not significant over total costs.

Inputs like conventional solvents affect the cost of the extraction processes and environment. Even that supercritical extraction requires a higher energetic cost, overall, it is cheaper than conventional ones.

The gathering and evaluation of studies analyzing the life cycle sustainability of microalgae lipid extraction systems is critical for the dissemination of microalgae systems, the utilization of their current potential, and the determination of future vision. As a result, it is recommended that future studies examine life cycle economic, social, and environmental sustainability.

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UTILIZATION OF INDUSTRIAL ROSE AND LAVENDER SOLID BY-PRODUCTS FOR REMOVAL OF 2-NAPHTOL ORANGE

Gergana MAROVSKA, Anton SLAVOV

Department of Organic Chemistry and Inorganic Chemistry, Technological Faculty, University of Food Technologies, 26 Maritsa Blvd, 4002, Plovdiv, Bulgaria

Corresponding author email: gerii_vlaseva@abv.bg

Abstract

Lavender (Lavandula angustifolia Mill.*) and rose (Rosa Damascena* Mill.*) industrial by-products were used as natural, biodegradable, readily available and cheap bioadsorbents to remove the azo dye 2-naphthol orange from waste waters. The influence of contact time, pH, adsorbent amount, initial dye concentration, temperature, sonication and adsorbent particle size was investigated. The acidic medium favored the adsorption and at pH 1.5, 99% efficiency (for lavender as adsorbent) was achieved. The results of the present study confirmed the successful application of essential oil industrial lavender and rose by-products as bioadsorbents for efficient removal of 2-naphtol orange from waste waters as a new method for valorization of the by-products from agricultural industry.*

Key words*: rose, lavender, by-products, 2-naphthol orange, adsorption.*

INTRODUCTION

Azo dyes are among the most widely used chemical class of dyes worldwide. They are synthesized from aromatic amines by diazotization (Crettaz et al., 2020). Azo dyes have a toxic effect on humans, as well as, on nature and aquatic life. Therefore, there is an urgent call for wastewaters containing azo dyes to be treated for their elimination or for their conversion into safer products (Benkhaya et al., 2020). Most of the dyes are mutagenic and carcinogenic, they are stable to light, temperature and biodegradation, which makes them risk-posing compounds. Therefore, effective methods to remove these substances from wastewaters are important. The methods used for the purification of the wastewaters are coagulation, flocculation, precipitation, membrane filtration, electrochemical techniques and conventional biological treatments (activated sludge). However, these techniques are not 100 percent efficient nor are they financially viable, as most of them require large land areas and are quite expensive (Kumar et al., 2011).

Adsorption is a purification process that is one of the most cost-effective methods for removing pollutants from wastewaters. It requires little space and the process is not affected by toxic chemicals. Adsorption processes also allow regeneration, recovery and recycling of the adsorbents (Nascimento et al., 2014). Besides, often by-products from agricultural industry (essential oil industry for example) could be used as natural, biodegradable, cheap and readily available adsorbents.

Hence, 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 the azo dye 2-naphthol orange.

MATERIALS AND METHODS

Lavender (L) (bio-certified *Lavandula angustifolia* Mill., 'Sevtopolis' var.) and rose (RD) (bio-certified *Rosa Damascena* Mill.), by-products from industrial steam distillation of fresh plant material were provided by the ECOMAAT distillery (Mirkovo, region of Sofia, Bulgaria; crop 2021).

2-naphthol orange (Sodium 4-[(2-hydroxynaphthalen-1-yl)-diazenyl] benzenesulfonate) was obtained from local distributors.

The rose and lavender by-products were collected from the still after the end of industrial treatment. The solid residues were inspected for impurities and dried. The dried rose and lavender were washed with distilled water (100 g with 1600 mL water; added in portions of 400 mL) on a Buchner funnel. The residual solid mass was dried, milled and sieved. The same conditions were used for washing of the RD and L by-products with acetone, 70% ethanol and 0.1 N hydrochloric acid. For adsorption of the azo dye, fraction with particle size 50-100 μm was used.

The adsorption of 2-naphthol orange was carried out as follow: 20 mL water solution of 2-naphthol orange with the specified concentration (20, 40, 60, 80 and 100 mg/L) were added to 1 g of adsorbent in a 50 mL centrifuge tube and the tubes were put on a laboratory shaker MLW THYS 2 (VEB MLW Labortechnik Ilmenau, Germany), placed in a thermally controlled laboratory oven. 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 mass was filtered first through a paper filter and further through a syringe filter CA 0.45 μm (Isolab, Germany). Adsorption of the filtrate was measured at 500 nm using LLG-uniSPEC 2 UV-Vis spectrophotometer (LLG Labware, Germany) and the concentration of the remaining after the adsorption 2-naphthol orange was calculated using a calibration curve, prepared with water solutions of the dye with known concentrations.

RESULTS AND DISCUSSIONS

Influence of the contact time on the adsorption of 2-naphthol orange

The first parameter investigated was the contact time of the adsorbent with the 2-naphthol orange. This parameter determines the dye adsorption time equilibrium and is important from economic and ecological point of view (Figure 1). In the beginning of the contact of the azo dye with the adsorbent, adsorption is a fast process since there are a lot of unoccupied sites in the surface of the L and RD adsorbents. Sharp increase of the adsorbed azo dye was observed until the 30th min, after that, clearly, equilibrium was observed. At 120 min the efficiency of the adsorption at pH 7.05 was 92% for RD and 85% for L as adsorbents for 2-naphthol orange. Similar observations were made by Wu et al. (2011) investigating adsorption of 2-naphthol orange using spent brewery's yeast. The authors found that the equilibrium of the adsorption was reached after 20-30 min contact time.

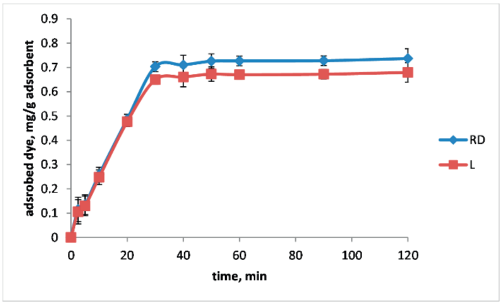

Figure 1. Influence of the contact time on the adsorption of 2-naphthol orange (initial concentration of the azo dye: 0.8 mg/20 mL; pH: 7.05; amount of the adsorbent: 1 g; temperature: 20ºC; shaking at 100 rpm)

Influence of the pH of the medium on the adsorption of 2-naphthol orange

Furthermore, the effect of the pH of the medium was investigated (Figure 2). The acidic medium favored the adsorption process and the highest effectiveness of the removal for both adsorbents was achieved at pH 3.5. At pH 1.05, however, the L residue was able to eliminate 99% 2-naphthol orange. At pH 10.75 almost no adsorption of 2-naphthol orange was observed. These results are supported by the findings of Wu et al. (2011) and Pelosi et al. (2013) investigating adsorption of 2-naphthol orange on waste biomasses. However, in the work of Wu et al. (2011) the effectiveness of the adsorption lowered significantly after pH was raised above 5. In our case both adsorbents RD and L could be utilized at pH levels up to 7. This observation is important because it is much easier to operate industrially at neutral pH than at acidic medium. Besides, further it will be necessary to neutralize the spent adsorbent and also treatment of the solution will be necessary.

The effectiveness at lower pH might be related to the net charge of the adsorbents. The main functional groups which could contribute to the adsorption are carboxyl and hydroxyl groups (Hamzeh et al., 2012). At lower pH medium these groups will be protonated and attraction will favor retention of the 2-naphthol orange. Due to its anionic character, at higher pH (when the carboxyl groups will not be protonated) electrostatic repulsion will lower the effectiveness of the adsorption.

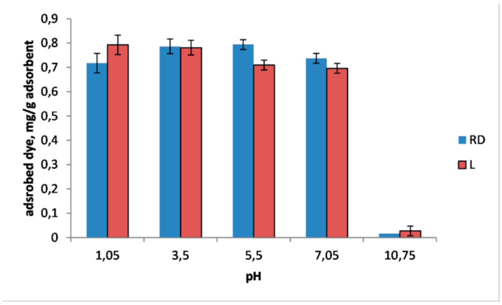

Figure 2. Influence of the pH on the adsorption of 2-naphthol orange (contact time 40 min; initial concentration of the azo dye: 0.8 mg/20 mL; amount of the adsorbent: 1 g; temperature: 20ºC; shaking at 100 rpm)

Influence of the amount of the adsorbent/re-adsorption on the adsorption of 2-naphthol orange

The amount of adsorbent plays important role and usually contributes to the better effectiveness in the percentage removal of the pollutant. The best results were achieved (Figure 3) using 1.5 and 2 g RD adsorbent (single adsorption; almost 99% removal was achieved). For the L residue these amounts lead to retention of the solution and it was impossible to separate the purified water.

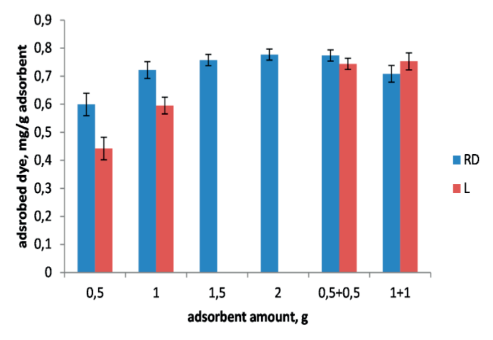

Figure 3. Influence of the amount of the adsorbent/re-adsorption on the adsorption of 2-naphthol orange (contact time 40 min; initial concentration of the azo dye: 0.8 mg/20 mL; pH: 7.05; temperature: 20ºC; shaking at 100 rpm)

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 were highly effective. One of the problems arising using consecutive adsorption was related to the higher water retention capacity of the RD and L, having highly hydrophilic character. At doses of 1.5+1.5 g adsorbents no water phase could be obtained after adsorption.

Influence of the initial concentration of the 2-naphthol orange on the adsorption

At all the investigated dye concentrations (0.02 to 0.1 mg/mL) the effectiveness of the adsorption achieved was around 90%. The highest percentage (92.74%) of adsorption of 2-naphthol orange (at 0.04 mg/mL concentration) has the RD adsorbent (Figure 4). This might suggest that the capacity of the adsorbents RD and L is much higher than 0.1 mg/mL per 1 g adsorbent.

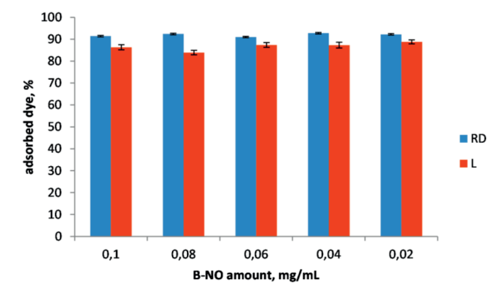

Figure 4. Influence of the concentration of the 2-naphthol orange on the adsorption (contact time 40 min; amount of the adsorbent: 1g; pH: 7.05; temperature: 20ºC; shaking at 100 rpm)

Influence of the temperature on the adsorption of 2-naphthol orange

In general the effect of temperatures above 20°C was negative on the effectiveness of adsorption for both adsorbents and this was more clearly expressed for L (Figure 5). This effect might be due to the increased rate of desorption with increase of the temperature.

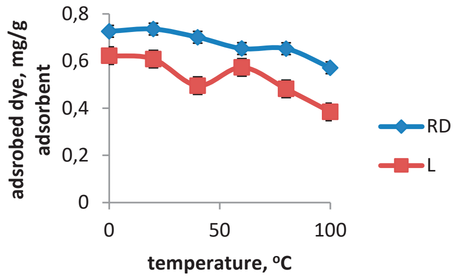

Figure 5. Influence of the temperature on the adsorption of the 2-naphthol orange (contact time 40 min; initial concentration of the azo dye: 0.8 mg/20 mL; amount of the adsorbent: 1 g; pH: 7.05; shaking at 100 rpm)

Influence of the particle size of the adsorbent on the adsorption of the 2-naphthol orange

In the next experiments the influence of the particle size of the adsorbents on the adsorption was investigated. Results presented in Figure 6 clearly demonstrated that for both adsorbents particles with a size of 50 μм or below had adsorbed most effectively the azo dye. This result is not surprising having in mind that the lower the particle size the more active surface the adsorbent will have.

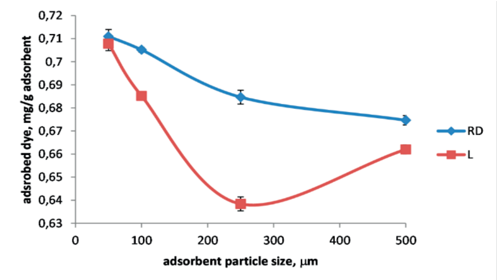

Figure 6. Influence of the particle size of the adsorbent on the adsorption of the 2-naphthol orange (contact time 40 min; initial concentration of the azo dye: 0.8 mg/20 mL; amount of the adsorbent: 1 g; pH: 7.05; shaking at 100 rpm)

Influence of the type of the adsorbent on the adsorption of the 2-naphthol orange

In the following experiments the RD and L were compared with several known adsorbents: activated carbon, Al_2O_3 and silicagel (Figure 7). The best results for adsorption effectiveness showed the activated carbon. RD and L showed better adsorption capacity than Al_2O_3 and silicagel and the percentage of removal of 2-naphthol orange was closer to that of the activated carbon. The type of the initial pretreatment of the rose and lavender by-products was also assessed. Both by-products were washed with 70% ethanol, acetone and 0.1 N hydrochloric acid (HCl), dried and used as adsorbents. The results suggested that acid washing was beneficial to the applicability of the residues as adsorbents. The best adsorption effectiveness showed both acid-washed rose and lavender adsorbents, as L_Res_AE adsorbed 0.7918 mg/mL of 0.8 mg/mL 2-naphthol orange (almost 99% removal of the dye). This corresponds well with the results obtained for the influence of the pH on the adsorption of the 2-naphthol orange (Hamzeh et al., 2012).

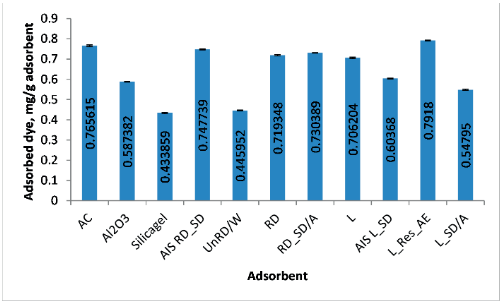

Figure 7. Influence of the type of the adsorbent on the adsorption of the 2-naphthol orange (contact time 40 min; initial concentration of the azo dye: 0.8 mg/20 mL; amount of the adsorbent: 1 g; pH: 7.05; shaking at 100 rpm). AC - active carbon; Al_2O_3; Silicagel; AIS RD_SD - rose by products washed 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

Influence of the ultrasound treatment on the adsorption of the 2-naphthol orange

Ultrasound treatment has gained popularity during the recent years with its simplicity and effectiveness for many industrial applications (Slavov et al., 2019). Ultrasound could be successfully applied for adsorption/desorption purification processes in the food industry (Wu et al., 2018), pollutant removal (Hamdaoui, et al., 2003), etc. Hence, in the next experiments the influence of the sonication on the removal of 2-naphthol orange was studied (Figure 8).

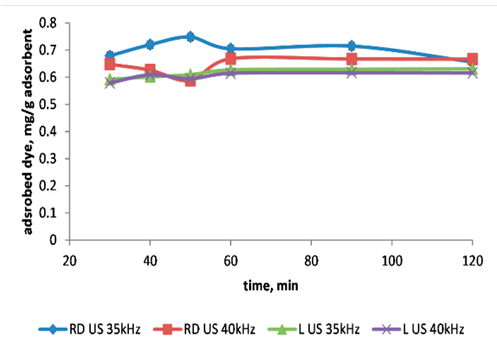

Figure 8. Influence of the ultrasound treatment on the adsorption of the 2-naphthol orange (contact time 40 min; initial concentration of the azo dye: 0.8 mg/20 mL; amount of the adsorbent: 1 g; pH: 7.05)

Two treatments - at 35 kHz and at 40 kHz frequency were investigated. It could be seen that equilibrium was established after around 20-30 min and the percentage removal was similar to the experiments for the contact time (without sonication) (Figure 1). This suggested that at the experimental conditions sonication did not improved significantly the removal by adsorption of the 2-naphthol orange. Similar observations were made by Hamdaoui et al. (2003) while investigating adsorption/ desorption process of *p*-chlorophenol on granular activated carbon. One possible explanation of the results obtained was that along with the adsorption intensification from sonication, the reverse process of desorption also occurred with time, when the adsorbent had already adsorbed its maximum azo dye.

The results from the experiments suggested that RD and L by-products could be successfully applied as a bioadsorbent for removal of the azo dye 2-naphthol orange from water solutions. The residues generated after adsorption of 2-naphthol orange 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 2-naphthol orange the higher fungi, able to produce lignocellulosic enzyme complexes, could successfully degrade the azo dye and by this way completely eliminate the hazardous threat for the nature.

CONCLUSIONS

The present study explored the application of two industrially generated by-products of the essential oil rose and lavender industry. The by-products are cheap, renewable, biodegradable and natural substances, which could be successfully applied for biosorption of organic pollutants, such as azo dyes. The influence of contact time, pH, amount of the adsorbent and 2-naphthol orange, particle size, temperature, initial pretreatment of the adsorbent and mechanical treatment were investigated. It was found that equilibrium was established after 20-30 min contact time, and that the lower pH favors the adsorption process. The increase of the temperature influenced negatively the removal of 2-naphthol orange and the best results were obtained at temperatures below 20°C. The adsorbents' particle size below 50 μм showed the best results for adsorption effectiveness. Acid washing (with 0.1 N HCl) as a pretreatment leads to the best results for removal of 2-naphthol orange from the medium. The resulted adsorbents had adsorption capacity higher than that of activated carbon as a well-known commercially available adsorbent. The best adsorption effectiveness showed both acid-washed rose and lavender adsorbents, as lavender acid washed by-product adsorbed 0.7918 mg/mL of 0.8 mg/mL 2-naphthol orange (almost 99% removal of the dye).

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ANTHROPOGENIC INFLUENCE ON THE PHYSICAL PROPERTIES OF SOILS IN GREENHOUSES AND SOLARIA

Casiana MIHUȚ, Anișoara DUMA COPCEA, Lucian NIȚĂ, Adalbert OKROS, Daniela SCEDEI, Daniel POPA, Carmen DURĂU, Teodor MATEOC-SÎRB

University of Life Sciences "King Mihai I" from Timișoara, 300645, 119 Calea Aradului, Romania, Phone: +40256277001, Fax:+40256200296

Corresponding author emails: casiana_mihut@usab-tm.ro, anisoaradumacopcea@usab-tm.ro

Abstract

The paper addresses the influence that man has on the physical properties of the soil. Thus, a series of physical properties of soils in greenhouses and solaria are compared, namely: texture, structure, density, apparent density, total porosity, and aeration porosity following the application of organic and mineral fertilisers. The studies and research were carried out in greenhouses and solaria located in the western part of Romania (Timiș and Arad counties), in two localities, Giarmata and Secusigiu, cultivated with tomatoes (Solanum lycopersicum), peppers (Capsicum annuum) and eggplant (Solanum menongena) over a period of 9 years. The physical properties of soils were compared in 2016, 2019, and 2022, and the results showed that, in the solaria in which organic fertilisers (manure) were applied in doses of 40-60 t/ha, density values decreased from 1.12 g/m^3 to 1.08 g/m^3, apparent density from 1.65 g/m^3 to 1.54 g/m^3, while total porosity values increased from 62% to 67% and aeration porosity from 36% to 41%, thus falling within normal values of vegetable cultivation.

Key words: *anthropic influence, physical properties, protected areas.*

INTRODUCTION

Lately, man has become an active factor in soil genesis, either directly - through improvement works or plant cultivation, or indirectly - through diking, drainage, irrigation, or erosion control works (Annabi et al., 2011). Following anthropic interventions, most of the soils have evolved, in the last hundred years, under intensely modified anthropic conditions (Grosbellet et al., 2016).

Over time, the influence of man on these processes has taken various forms with different intensities, causing, in all cases, the disturbance of the natural conditions of soil genesis and, in some cases, leading to their improvement (Canarache, 1997; Mihuț et al., 2018).

The positive activity of man in the evolution of soil and its fertility is also materialized through amendments and fertilizers (Okros A., 2015)

By correctly applying the fertilizers, not only the crops, but also the suitable properties of the soils increase, for example, tri-calcic phosphates enrich the cationic component of the colloidal complex, and organic fertilizers increase the microbiological activity, as well as its porosity. However, irrationally applied organic fertilizers can create pollution situations in the soil and even imbalances in plants (Goian & Ianoș, 1993).

Agricultural wastes can be used as a source of organic matter and nutrients for soils and influence the physical properties of soils. They can also be easily applied as mulching, providing numerous advantages (Rico et al., 2016). So, this chapter gives an overview of the positive effects of recycling vegetable wastes and soil physical properties (Diacono et al., 2010).

Soil structure is one of the most important soil's physical factors controlling or modulating the flow and retention of water, solutes, gases, and biota in agricultural and natural ecosystems (Lal et al., 1991; Young et al., 1998). Soil structure is very important in soil productivity and is a limiting factor of crop yield (Cotching, 2018).

The physical properties of soils condition their quality and, in particular, the porosity which affects different processes related to the transformations of organic matter, gas exchange, the growth of plant roots, and movement of water in the soil, as before it was indicated (Archer et al., 2006).

Soil porosity is the property that, due to the effect of compaction, is being altered largely in the European Union (and developing countries), together with the loss of organic matter from soils, and, for this reason, our management of the soils should allow maintaining this property at adequate levels (European Commission, 2015).

Bulk density is an important indicator of soil quality, productivity, compaction, and porosity. BD is mainly considered to be useful to estimate soil compaction. Root length density, root diameter, and root mass were observed to decrease after an increase in BD (Dal Ferro, 2014). However, the interpretation of BD with respect to soil functions depends on soil type, especially soil texture and soil organic matter (SOM) content (Six, 1998).

The two locations in which the research was carried out are in the western part of Romania, in Giarmata: it is in the central-northern part of Timiş County, 11 km from Timişoara, 1.3 km from Timişoara International Airport; Secusigiu is in Arad County and is located south of the Mureș river, on CR Arad-Sânnicolau Mare (Țărău et al., 2007; Mircov et al., 2021).

MATERIALS AND METHODS

The research was carried out in 8 protected areas, 4 on each location in Timiș and Arad counties, in two locations, Giarmata and Secusigiu, cultivated with tomatoes (*Solanum lycopersicum*), bell peppers (*Capsicum annuum*) and eggplant (*Solanum menongena*) for a period of 9 years. The soil is of the anthropic chernozem type following fertilization in Secusigiu, and of the vertic preluvosol in Giarmata, to which river sand was added in 2014, when the 4 solaria were built.

Considering that, in these protected areas, a series of vegetables are cultivated (3 crops/year), it is necessary to apply higher amounts of fertilizers. Fertilizers (organic and mineral) were applied, namely, manure in doses of 20, 40, and 60 t/ha, every 3 years; as mineral fertilizers, by irrigation, Solfert was used, in a doses of 10 kg/ha.

The first phase of the study was based on the method of observation and description. Such activities were carried out in the two locations with surprisingly different aspects of the soils. Soil samples were collected in the spring, before the basic crop.

During the 9 research years, a series of soil samples were collected from the 0-20 cm profile both in natural and disturbed settings and laboratory analyses of the following physical features of the soil were performed:

- Soil texture (%) was determined with the Cernikova method;
- Soil density (SD-g/cm^3) was determined with the picnometer and by estimation depending on the amount of humified organic matter and on the percentage of granulometric fractions;
- Soil apparent density (AD - g/cm^3) was determined with metal cylinders with known volume in natural setting;
- Total porosity (Tp %) was determined by calculus Tp = (1 - AD/SD) x 100;
- Aeration porosity (Ap%) was determined by calculus Ap = Tp – CC x AD.

RESULTS AND DISCUSSIONS

The research was carried out in two locations (Giarmata, Timiș County, and Secusigiu, Arad County) in 8 protected areas (4 in each location), the area of a solarium being between 400 and 500 sqm. In each of the two localities, two solaria were cultivated with tomatoes (*Solanum lycopersicum*), one with bell peppers (*Capsicum annuum*) and one with eggplant (*Solanum menongena*).

In the 4 solaria in each location, the same dose of fertilisers was applied, the determination of physical features was done every 3 years, i.e., in 2016, 2019, and 2022. As it appears from the data presented in Tables 1 and 2 and Figures 1 and 2, there are no high differences between the granulometric fractions of the soil texture in the two locations.

Table 1. Influence of the anthropic factor on soil texture in Giarmata

Nr. Crt.	Particle size fraction (%)	Year 2016				Year 2019				Year 2022			
		g.g.20 th/a	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g.20 t/ha	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g.20 t/ha	g.g. 40 t/ha	g.g. 60 t/a	Solfert 10 kg/ha
1.	Coarse sand (2.0-0.2 mm)%	15,9	15,8	15,9	15,9	16,0	16,1	16,3	16,1	15,7	16,0	16,4	15,8
2.	Fine sand (0.2-0.02 mm) %	37,7	37,8	38,0	37,7	37,8	37,4	37,5	37,6	37,6	37,6	38,0	37,7
3.	Dust (0.02-0.002 mm) %	25,3	25,4	25,5	25,3	25,4	26,2	26,9	25,3	25,5	25,5	25,6	25,4
4.	Clay (below 0.002 mm) %	21,1	21,00	20,6	21,1	20,8	20,3	20,2	21,0	21,2	20,8	20,0	21,10

Table 2. Influence of the anthropic factor on soil texture in Secusigiu

Nr. Crt.	Particle size fraction (%)	Year 2016				Year 2019				Year 2022			
		g.g.20 th/a	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g.20 t/ha	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g.20 t/ha	g.g. 40 t/ha	g.g. 60 t/a	Solfert 10 kg/ha
1.	Coarse sand (2.0-0.2 mm)%	1,3	1,4	1,5	1,3	1,3	1,5	1,6	1,3	1,4	1,6	1,7	1,3
2.	Fine sand (0.2-0.02 mm) %	19,8	20,1	21,2	19,7	19,7	20,8	21,6	19,6	21,0	21,8	22,2	19,6
3.	Dust (0.02-0.002 mm) %	46,6	4,8	47,2	47,0	46,8	47,0	47,8	47,2	47,3	48,2	48,3	47,2
4.	Clay (below 0.002 mm) %	32,3	31,7	30,1	32,0	32,2	30,7	29,0	31,9	30,3	29,4	27,8	31,9

The application in higher amounts (40-60 t/ha) of manure has led to a slight increase in sand and dust content especially in Giarmata, where the soil was modified by adding river sand, which finally led to modifying the texture from medium clayey-loamy to clayey, thus ensuring the most suitable conditions for the growth and development of vegetables.

In Secusigiu, the texture of the soil is medium. In the variants in which higher doses of manure were applied, the fine sand values increased from 1.3 to 1.7% when 60 t/ha of manure were applied. Fine sand had values between 19.6% in 2022 (Solfert variant 10 kg/ha) and 22.2% when 60 t/ha of manure was applied (2022). Dust values varied between 46.6% and 48.3% and those of clay, between 27.8% and 32.3%.

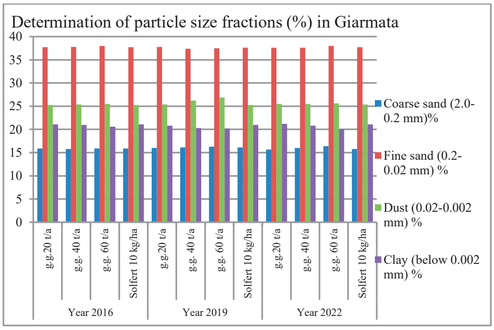

Figure 1. Influence of the anthropic factor on soil texture in Giarmata

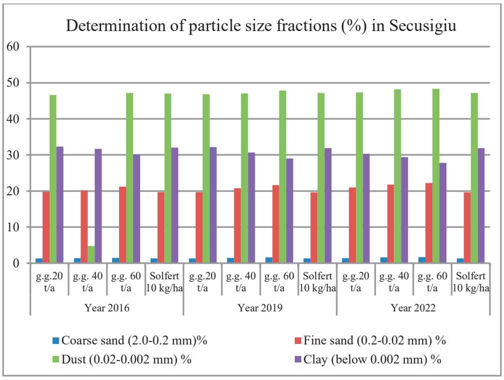

Figure 2. Influence of the anthropic factor on soil texture in Secusigiu

It can be stated that the texture of the soil did not change following the application of fertilizers since the values in the two locations were similar in all 4 solaria. There were little changes in the percentage content of the fraction of coarse sand and fine sand in the variants fertilised with higher amounts of manure, especially when applying a dose of 60 t/ha of manure.

The values of soil density and of apparent soil density in the two localities are presented in Tables 3 and 4 and in Figures 3 and 4.

Table 3. Influence of anthropic factor on soil density and apparent soil density (g/cm^3) in Giarmata

Nr. Crt.	Values obtained	Year 2016				Year 2019				Year 2022			
		g.g. 20 th/a	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g. 20 t/ha	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g. 20 t/ha	g.g. 40 t/ha	g.g. 60 t/a	Solfert 10 kg/ha
1.	Soil density (D - g/cm^3)	2.60	2.58	2.52	2.61	2.58	2.54	2.48	2.61	2.56	2.50	2.48	2.62
2.	Apparent density (DA - g/cm^3)	1.26	1.24	1.18	1.28	1.22	1.16	1.10	1.30	1.12	1.09	1.08	1.32

Table 4. Influence of anthropic factor on soil density and apparent soil density (g/cm^3) in Secusigiu

Nr. Crt.	Values obtained	Year 2016				Year 2019				Year 2022			
		g.g.20 th/a	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g.20 t/ha	g.g. 40 t/ha	g.g. 60 t/ha	Solfert 10 kg/ha	g.g.20 t/ha	g.g. 40 t/ha	g.g. 60 t/a	Solfert 10 kg/ha
1.	Soil density (D – g/cm^3)	2.48	2.40	2.28	2.60	2.46	2.38	2.26	2.61	2.42	2.28	2.18	2.62
2.	Apparent density (DA – g/cm^3)	1.36	1.32	1.28	1.38	1.32	1.26	1.20	1.38	1.28	1.24	1.16	1.40

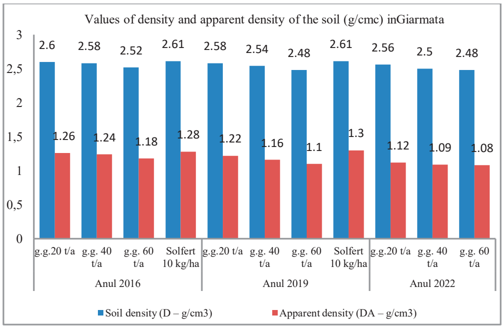

Figure 3. Influence of anthropic factor on soil density and apparent soil density (g/cm^3) in Giarmata

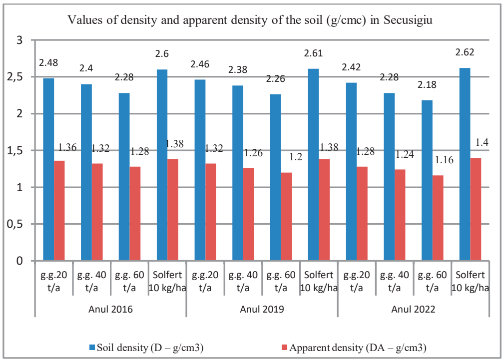

Figure 4. Influence of anthropic factor on soil density and apparent soil density (g/cm^3) in Secusigiu

The density of the soil in Giarmata had values between 2.62 g/cm^3 in the case of Solfert application in 2022, and 2.48 g/cm^3 in case of application of organic fertilization (manure 60 t/ha).
Soil apparent density had values of 1.09 g/cm^3 in the g.g. 60 t/ha and 1.32 g/cm^3 in the variant in which Solfert was applied in doses of 10 kg/ha.

In Secusigiu, the values of soil density and of soil apparent density were lower: 2.62 g/cm^3 in the mineral fertilized variant in 2022, and 2.18 g/cm^3 in the variant fertilized with 60 t/ha of manure also in 2022, when soil apparent density values were between 1.16 g/cm^3 and 1.40 g/cm^3. Higher values were found in the variants where Solfert was used in the organically fertilized variants. The higher the amount of manure, the lower the soil density and soil apparent density values.
Lower values were recorded in organically fertilized variants, and higher values in variants where mineral fertilizers were applied through fert-irrigation.
Tables 5 and 6 and Figures 5 and 6 present the values of total porosity and of aeration porosity in the two locations.
In Giarmata, total soil porosity had low values of 44-45% in the variants fertilised with Solfert by fert-irrigation and high values of 58% for the variant fertilised with 60 t/ha of manure (2022). Aeration porosity had values between 12-16% in the variants in which manure was applied in doses of 60 t/ha and between 9.20-9.40% in the variants in which Solfert was applied.
In Secusigiu, total porosity had values between 40-42% in the variants fertilise with Solfert, and 46-55% in the organically fertilized variants. Aeration porosity had values between 9.2-12.10% in the variants in which organic fertilizers were applied and between 8.7-8.90% in the variants in which mineral fertilizers (Solfert) were applied.

Table 5. Influence of anthropic factor on total porosity and total aeration porosity (%) in Giarmata

Nr. Crt.	Values obtained	Year 2016				Year 2019				Year 2022			
		g.g.20 t/a	g.g. 40 t/a	g.g. 60 t/a	Solfert 10 kg/ha	g.g.20 t/a	g.g. 40 t/a	g.g. 60 t/a	Solfert 10 kg/ha	g.g.20 t/a	g.g. 40 t/a	g.g. 60 t/a	Solfert 10 kg/ha
1.	Total porosity (PT %)	47	48	50	46	49	52	54	45	50	54	58	44
2.	Aeration Porosity (PA%)	9.80	10	12	9.60	10.2	14	15	9.40	14	15	16	9.20

Table 6. Influence of anthropic factor on total porosity and total aeration porosity (%) in Secusigiu

Nr. Crt.	Values obtained	Year 2016				Year 2019				Year 2022			
		g.g. 20 t/a	g.g. 40 t/a	g.g. 60 t/a	Solfert 10 kg/ha	g.g. 20 t/a	g.g. 40 t/a	g.g. 60 t/a	Solfert 10 kg/ha	g.g. 20 t/a	g.g. 40 t/a	g.g. 60 t/a	Solfert 10 kg/ha
1.	Total porosity (PT %)	46	48	50	42	47	49	52	40	48	51	55	40
2.	Aeration Porosity (PA%)	9.20	9.72	10.40	8.90	9.80	10.80	11.50	8.80	10.30	11.90	12.10	8.70

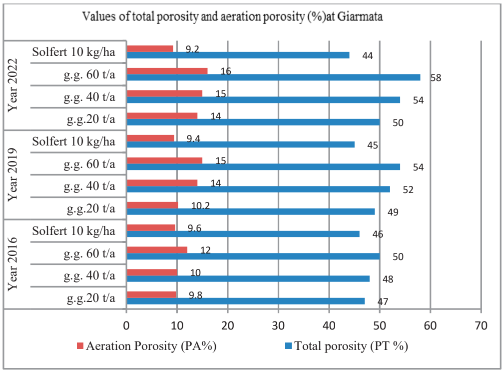

Figure 5. Influence of anthropic factor on total porosity and total aeration porosity (%) in Giarmata

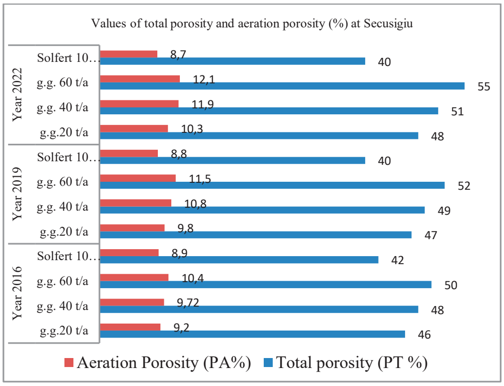

Figure 6. Influence of anthropic factor on total porosity and total aeration porosity (%) in Secusigiu

CONCLUSIONS

Following the research conducted in Giarmata and Secusigiu, the following conclusions can be drawn:

- Following the anthropic intervention on the vertic preluvosol in Giarmata, its initial features were significantly modified, especially on the surface, in the processed horizon (0-20 cm), in the sense that the content in fine and coarse sand increased and clay content decreased, because of both the application of manure in high doses (40-60 t/ha) and the administration of river sand in the amount of 10 t/ha;
- In Secusigiu, the chernozem has a medium clay texture, the variants fertilised with manure had a content in fine sand between 1.3% and 1.7% in the variant in which 60 t/ha of manure was applied, fine sand had values between 19.6% and 22.2%, dust had values between 46.6% and 48.3%, and clay had values between 27.8% and 32.3%;
- Soil density in Giarmata had values between 2.62 g/cm^3 and 2.48 g/cm^3 and soil apparent density had values between 1.09 g/cm^3 and 1.32 g/cm^3;
- In Secusigiu, the values of soil density were between 2.62 g/cm^3 and 2.18 g/cm^3 and those of apparent density, between 1.16 g/cm^3 and 1.40 g/cm^3;
- The highest values were recorded in the variants where Solfert was used and the lowest values were recorded in the organically fertilized variants.
- Total sol porosity in Giarmata had values between 44-58% and aeration porosity had values between 9.20-16%;
- In Secusigiu, total porosity had values between 40-55% and aeration porosity values were between 8.7 and 12.10%.

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CONSERVATION OF PLANT GERMPLASM USING SYNTHETIC SEED TECHNOLOGY - REVIEW

Ioana-Cătălina NICOLAE[1, 2], Oana VENAT[2], Adrian George PETICILĂ[1], Dorel HOZA[1]

[1]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: oana.venat@qlab.usamv.ro

Abstract

Plants are an essential resource for the existence of our planet. They sustain the ecosystems, produce the oxygen that is mandatory for the life of the other species and they are a source of fibres, medicine, materials and, the most important, they are our main source of food. In recent years, plant species are more and more threatened with extinction, due to urbanization, the development of various industries and habitat destruction. In addition to direct anthropological factors, natural habitats are endangered by climatic changes, changes that result in natural disasters as floods, bush fires, extreme temperatures and irregular precipitations. Conserving the plant species in in situ conditions, botanical gardens or field conditions exposes the germplasm to the risk of loss due to all these climatic accidents, and in vitro conservation techniques, such as synthetic seeds can provide a valuable solution for the future of our plant resources.

Key words: *artificial seed, encapsulation, germplasm, in vitro, micropropagation.*

INTRODUCTION

The Convention on Biological Diversity defines *in situ* conservation as the "conditions where genetic resources exist within ecosystems and natural habitats, and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties" and *ex-situ* conservation as "the conservation of components of biological diversity outside their natural habitats" (UNCED, 1992). *In vitro* conservation methods include conservation through slow growth, for short and medium-term storage and cryopreservation for long-term conservation (Engelmann, 1999). The slow growth conservation implies that the plant material is being conserved under growth-limiting conditions, such as lower temperature, lower light intensity, or by adding osmotic agents or growth retardants in the medium, with the aim to reduce the physiological processes and growth rate of the explants (Englemann, 1999). Trejgell et al. (2015) observed that the presence of ABA (in a concentration of 3.8 or 9.5 μM) in the conservation medium stimulated the survival and regeneration of *Senecio macrophyllus* shots conserved using slow growth storage technique. Benelli et al., 2022 listed some species stored through slow growth technique: *Castanea sativa* with 48 months storage time at 8°C, *Fragaria spp*. 15 to 18 months at 4°C, *Malus* spp. at 4°C from 18-20 to over 36 months with the addition of 2% mannitol and MS modified medium, *Pyrus* spp. at 4°C, from 12 to 48 months, depending on media formulation. Genebanks have a major role in germplasm conservation and ensure that genetic resources are protected and continuously available (FAO, 2014). The US Potato Genebank (Sturgeon Bay, Wisconsin) is holding the national collection of germplasm for *Solanum tuberosum*, a number of approximately 6000 accessions of 100 species of or related to *Solanum tuberosum,* one of the most important species of vegetables (Bamberg et al., 2016). Cultivars at the US Potato Genebank are cultured in small 20 mm x 150 mm glass tubes with 10 ml of medium and 3% D-sorbitol after being multiplied by axillary bud cutting. After a period of two weeks of establishment and growth at 20-22°C, they are moved into a chamber for long-term preservation, at 8-10°C, with lower light intensity, where those plants can remain viable for 1-1.5 years (Bamberg et al., 2016).

Figure 1. Example of the growing chamber for in vitro conservation of *Solanum* clones at US Potato Gene Bank (source: Bamberg et al., 2016)

According to the website of the International Potato Center (CIP), headquartered in Lima, Peru, this genebank was founded in 1971 and after 45 years of continuous focus on germplasm collection to eliminate mixtures, atypical plants, and plants with virus symptoms, from 17.347 ascensions, with duplicate removal, the nowadays potato collection includes 4870 accessions with 4467 traditional landrace cultivars from 17 countries (mainly from the Andean region) and improved varieties. The entire collection is conserved by *in vitro* techniques and all distribution is made by tissue culture material. Plants are continually reevaluated to identify duplicates, verify identity, and maintain accurate morphological, taxonomic, and cytogenetic characterization. Potato is a species optimized for long-term cryopreservation, so there are over 450 new ascensions entered into this procedure over one year. Regarding the sweet potato germplasm collection, the International Potato Center (CIP) has one of the world's largest cultivated sweet potato gene banks, with over 5500 accessions. All material is *in vitro* maintained and the main CIP objective for this seventh most worldwide important food crop is to conserve the collection biodiversity and make it available for breeding research and training, as they mentioned on their public page.

Maintaining germplasm *in vitro* cultures is labor intensive, time-consuming and exposes the plant material to the risk of losing the germplasm due to contamination or increase of somaclonal variations, even in the case of using slowed/reduced growth conditions (Pannis et al, 2001). To overcome this, synthetic seed technology may be applied to conserve species on short and medium time and cryopreservation for long-term preservation. Synthetic seeds are represented by somatic embryos, axillary or terminal buds, nodal segments, cell aggregates, or other types of artificially encapsulated tissues that can be used for sowing and can transform into plants, and that can retain this ability even after short term and medium-term storage (Hussain et al., 2000; Micheli & Standardi, 2016; Magray et al., 2017). The concept of synthetic seed was first introduced in 1977, by Murashige. Initially, the term referred only to encapsulated somatic embryos (Murashige, 1977), but later, the concept was extended to non-embryogenic tissues as well (Bapat et al., 1987). The technology of synthetic seeds has many applications: it can be used to asexually propagate endangered species, and valuable genotypes of species that normally don't produce seeds (Qahtan et al., 2019). The technique can also be used for exchange between laboratories and institutions and short and medium conservation of germplasm (Standardi a Micheli, 2013). Long-term conservation of synthetic seeds can be achieved by cryopreserving the plant material. Otherwise, these can be stored for short and medium time in the fridge, at 2-8°C, depending on the species (Micheli & Standardi, 2016).

Synthetic seeds can be stored up to 90 days in the fridge, but the optimum storage period is dependent on the species, but generally, most species can be stored in the fridge, at 4-6°C (Qahtan et al., 2019). Some species can be stored at room temperature, in dark conditions (Standardi & Micheli, 2013). High humidity and low temperature are essential for storing the synthetic seeds (Mallikarjuna et al., 2016). In terms of plant genetic resources conservation, cryopreservation is one of the safest methods. Once a cryopreservation method is established for each species, the germplasm can be stored for a very long period, without the need for further transfers or replanting (Bamberg et al., 2016). Even though the initial cost of storing the plant material in liquid nitrogen is high, the cost of culture maintenance in LN is lower than storing them as field cultures or as *in vitro* cultures (Bamberg et al., 2016).

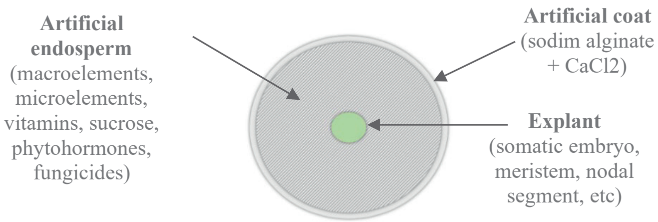

Figure 2. Structure of synthetic seeds

Types of explants used for encapsulation: Somatic embryos

The advantage of using somatic embryos for synthetic seeds is that these types of explants have a bipolar structure and can develop roots and shoots simultaneously, without the need for further auxin treatment (Hussain et al., 2000). The use of somatic embryos in the synthetic seed technology was studied in numerous species, such as *Rotula aquatica* (Chithra et al., 2005), *Oryza sativa* (Kumar et al., 2005), *Pinus patula* (Malabadi & Staden, 2005), *Pinus radiata* (Aquea et al., 2008), *Vanda tessellate* (Manokari et al., 2021), *Quercus suber* L. (Pintos et al., 2008), *Litchi chinensis* Sonn (Das et al., 2016). Malabadi and Staden (2005), obtained a regeneration rate of 89 % for encapsulated somatic embryos of *Pinus patula.* The synthetic seeds were conserved at 2°C, for 120 days and did not register a significant decrease regarding viability, compared to the embryos that were not encapsulated, where the viability decreased to 2%, after only 20 days of storage at 2°C.

Holobiuc and Catana (2012), developed a protocol for somatic embryogenesis and synthetic seed production for *Gentiana lutea,* a species regarded as critically endangered in Romania. Studies by Kamińska et al., 2017, indicate that synthetic seeds of *Taraxacum pieninicum*, a critically endangered species of the Asteraceae family, can be stored for up to 12 months at 4°C, with successful recovery and without any genetic variations. *Vanda tessellata*, an epiphytic orchid whose roots are valued in traditional medicine (Hossain, 2011; Shengji and Zhiwei, 2018) can be stored for 12 months at -4°C, and then regenerated on medium with 0.5 mg/L BAP (6-(benzylaminopurine), 0.5 mg/L KIN (kinetin) and 0.5 mg/L IAA (Indole-3-acetic acid) (Manokari et al., 2021).

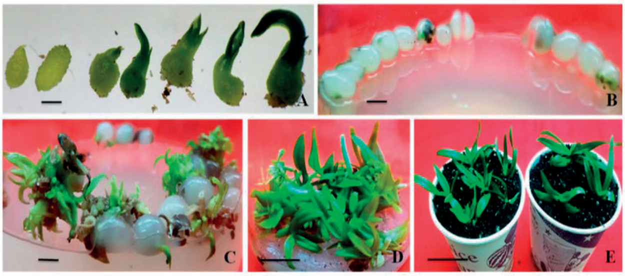

Figure 3. Regeneration of *Vanda tessellata* using encapsulated somatic embryos: A. various developmental stages of SE; B. inoculation and emergence of shoots; C. germination and emergence of shoots; D. multiplication and elongation; E. rooted plantlets (source: Manokari et al., 2021)

Protocorm-like bodies (PLBs)

Sarmah et al. (2010) successfully conserved encapsulated PLBs of *Vanda coerulea* for 100 days in cold storage, with a conversion rate of 94.9%. Encapsulated PLBs of *Flickingeria nodosa* can be stored for 3 months at 4°C with a regeneration rate of 95% (Nagananda et al., 2011).

Meristematic tissues. Apical and axillary buds. Nodal segments

Because of the limits that somatic embryos possess, in 1978, Bapat proposed the use of non-embryogenic tissues for encapsulation as synthetic seeds, especially in the case of species that are recalcitrant to somatic embryogenesis. Generally, these types of tissues are easier to obtain compared to somatic embryos, and the risks of somaclonal variations are highly reduced. In addition, the use of non-embryogenic tissues for synthetic seeds can be used for the vast majority of species (Standardi & Micheli, 2013).

Apical and axillary buds can easily regenerate plants from synthetic seeds, as long as these explants have a source of nutrients and if their the rooting process is not problematic. In many species, the regeneration of shoots and rizogenesis happen simultaneously (Gantait et al., 2015). In 1990, Bapat and Rao obtained *Morus indica* plantlets from synthetic seeds sown *in vivo*, without auxin treatments. Ganapathi et al., 1993, obtained 100% regeneration of *Musa* synthetic seeds sown on White's medium, without any treatment for rhizogenesis. In the case of using apical and axillar buds in species that have difficulties rooting, it is recommended that auxin treatments are applied to induce the process (Hussain et al., 2000). Capuano et al., 1998, obtained *Malus domestica* rootstock 'M26' plantlets from encapsulated apical and axillary buds using IBA in a concentration of 24.6 µM.

Shoot tips are frequently used as explants in the production of synthetic seeds. Explants can be relatively easily produced and these have the capacity to retain their viability and regeneration potential for a relatively long time (Piccioni & Standardi, 1995). Shoot tip encapsulation was studied in numerous species, such as *Solanum tuberosum* (Ghanbarali et al., 1995), *Ruta graveolens* (Ahmad et al., 2012), *Glochidion velutinum (*Mallikarjuna et al., 2016*), Helianthus annus* L. *(*Katouzi et al., 2011*), Vitis vinifera* (Benelli, 2016), *Begonia spp* (Sakhanokho et al., 2013), *Mentha arvensis* L. (Islam & Bari, 2012). Benelli (2016) encapsulated nodal segments and shoot tips of *Vitis vinifera* rootstock 'Kober 5BB' and after a storage period of 9 months at 4°C and dark conditions, the encapsulated nodal segments and shoot tips had regeneration rates of 55.6% and 83.3%, respectively. Katouzi et al. (2011) encapsulated shoot tips in alginate solutions containing different concentrations of salicylic acid and studied the viability of the explants after 90 days of cold storage. The addition of salicylic acid in the encapsulation matrix may increase the explant tolerance to cold temperatures during storage. The addition of 25 µM and 50 µM salicylic acid proved to have a beneficial effect on viability of the seeds and increased the viability rates to 48%, respectively 59%.

Callus cells

As a source of explant for synthetic seed production, callus cells are the least used The undifferentiated nature of these cells and the special conditions required for the differentiation of cells can be problematic when used for synthetic seeds (Gantait et al., 2015). Kim and Park (2007) regenerated *Allium sativum* plants from callus cells encapsulated in a solution of halved concentration MS salts, 1.5% sodium alginate, 3% sucrose, kinetin, NAA, and 50 mM $CaCl_2$. The encapsulated cells had a regeneration rate of 88%, after 40 days of storage at 4°C. Zych et al. (2005), successfully encapsulated differentiated callus cells of *Rhodiola kirilowiiI.* The percentage of developed shoots stored for 1-6 weeks at 4°C was registered between 95% and 100%.

In vitro* conservation of *Solanum tuberosum

Storing potato tubers requires growing them annually in nurseries, an operation that is time-consuming and exposes the vegetal material to pests, diseases, and climatic accidents (Roque-Borda, 2021). Conserving the germplasm in this species through true potato seeds is not possible, as this species is highly heterozygous and the seeds produced are not true to type (Dodds et al., 1991).

Conservation using synthetic seed technology was achieved using nodal segments (Sarkar and Naik, 1998; Patel et al., 2000), axillary buds (Ghanbarali et al., 2016), shoot tips (Nyende et al., 2003; Patel et al., 2000) and somatic embryos (Maid et al., 2010; Fiegert et al., 2000), call (Patel et al., 2000).

Ghanbarali et al. (2016) concluded that the optimum beads were obtained using 3% sodium alginate and 1% $CaCl_2$ for the encapsulation matrix. Concentrations of Na-alginate lower than 3% produced capsules that were too fragile and difficult to handle, whereas at concentrations higher than 3%, capsules were too hard and the regrowth speed of the encapsulated buds was delayed. Nyende et al. (2003) investigated the encapsulation of *S. tuberosum* shoot tips in calcium alginate hollow beads. Sarkar and Naik, 1998, encapsulated *in vitro* derived nodal segments for potato propagule production, with direct sowing into the soil, in ex vitro conditions, with a 57% rate of survival in soil. The encapsulated segments were incubated for 3 days and treated with rooting powder at the time of planting.

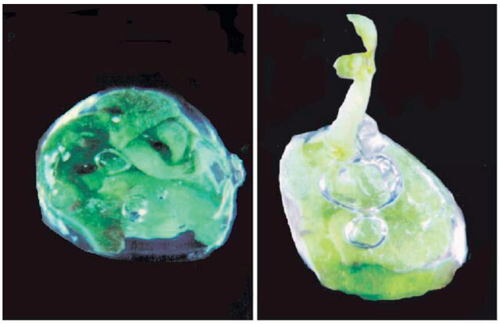

Figure 4. Synthetic seeds of *Solanum tuberosum;* left: before germination and right: after germination (Source: Bernand et al., 2002)

Nyende el al. (2003), concluded that shoot tips of *S. tuberosum* can be stored with a 100% regeneration rate for 180 days at 4°C and 10°C and at for 270 days at 4°C. Increasing the storage time to 360 days at 4°C, the recorded viability rates dropped between 70.8% and 51.5% depending on the cultivar that was encapsulated in the beads. It was also observed that shoot tips progressively turned brown during storage, faster at 10°C than at 4°C.

Ghanbarali et al. (2016) conserved *S. tuberosum* synthetic seeds for 90-120 days at 4°C without loss of viability, depending on the cultivar, using explants that were initially precultured on MS medium with 10-6 M 24-epibrassinolide for 2 days, which might improve the tolerance to cold temperatures. Preculture of the shoots in medium containing 10-6 M 24-epibrassinolide positively influenced the regrowth rate, speed, and shoot length for both the cultivars studied ('Sante' and 'Agria'). 24-epibrassinolide (EBr) is a plant growth regulator from the brassinosteroids class and influences a range of processes of growth and development (Ghanbarali et al., 2016) and tolerance to different types of abiotic stresses: salt stress (Alam et al., 2019), high temperatures (Dhaubhadel et al., 1999; Singh and Shono, 2005; Pociecha et al., 2017).

The advantages of these type of seeds are that, like seeds obtained from sexual propagation, they are easy to store, as they don't need a lot of space or special conditions, easy transportation, the possibility of using sowing equipment are the sterile environment in which they are stored provides protection from pests and diseases. Moreover, cryopreservation can be applied to increase the storage capacity of the encapsulated explants (Lambardi et al., 2006). Synthetic seeds, unlike orthodox seeds, don't need a dormancy period for germinating and are genetically identical to the mother plant (Saxena et al., 2019).

CONCLUSIONS

The risk of losing our biodiversity is increased nowadays, as more and more species are threatened with extinction, due to both climatic and anthropological factors, and conservation of plant germplasm is crucial. Traditional conservation in botanical gardens or field collections still exposes germplasm to threatening factors and *in vitro* methods become more and more reliable for plant conservation. But these methods generally come with a risk of somaclonal variations, and contamination, and they are time-consuming and manual labor is required, subcultures need to be reduced to a minimum. Synthetic seed technology combines the advantages of clonal propagation with sexual propagation, as these are easy to store, to transport, sowing equipment can be used, they are protected against pests and diseases and they produce plants identical to the mother plant. Numerous studies have shown that synthetic seed technology can aid *in vitro* conservation of plant germplasm.

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WATER STRESS MANAGEMENT USING HYDROGELS AND THEIR IMPACT ON PLANT BIOCHEMICAL COMPOSITION OF *LACTUCA SATIVA*

Ion NIȚU[1], Silvana DĂNĂILĂ-GUIDEA[1], Elena MĂNĂILĂ[2], Elisabeta Elena POPA[1], Mihaela GEICU-CRISTEA[1], Mihaela Cristina DRĂGHICI[1], Paul Alexandru POPESCU[1], Mona Elena POPA[1], Amalia Carmen MITELUȚ[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnologies, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Institute for Laser, Plasma and Radiation Physics, Magurele, Romania

Corresponding author emails: elena.eli.tanase@gmail.com, mihaela_geicu@yahoo.com

Abstract

In the recent decades, hydrogels have proven great potential for agricultural usage regarding water management and controlled release of stimulants and further improving the food production. The aim of this study is to analyse the effects of 4 different hydrogel compositions in 2 forms of administration (beads and granules) on water management and the impact on the biochemical composition of lettuce (Lactuca sativa). In this regard, two different conditions were assured, namely induced drought stress and regularly irrigated conditions. The lettuce plants were periodically analysed to determine the ascorbic acid content, polyphenolic content and antioxidant activity. The results showed slightly higher content in ascorbic acid for the regularly irrigated plants. Contrary, the antioxidant activity and total polyphenolic content showed an increase in plants subjected to drought stress. Between the two forms of hydrogel administration, the granular form presented better properties in alleviating the water stress induced by the extensive period of drought.

Key words: *biochemical parameters, hydrogels, water stress.*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most consumed and farmed leafy vegetable in the world, with output increasing year after year. In 2018, the total harvested area was anticipated to be more than 1.27 million hectares, with a total yield of roughly 27.3 million tons (Medina-Lozano et al., 2021).

Due to water shortage, irrigation water conservation is becoming increasingly critical in lettuce output to achieve optimal yield. Furthermore, due to rising human demand, water supplies for agriculture is sometimes insufficient. Given the increased focus on the efficient use of precious water resources, it is critical to enhance water usage efficiency (Chen et al., 2019). Drought has a negative influence on plant development, growth, and fertility. Long-term drought stress and increased stress severity result in further acclimation processes. These responses include osmotic adjustment, reduced shoot-root ratio, cell wall changes, metabolic reprogramming, and antioxidant system activation (Laxa et al., 2019).

Hydrogels have developed as an efficient technology for increasing soil water-holding capacity and conserving moisture, particularly in dry and semi-arid environments. These crosslinked polymers, known as hydrophilic gels, absorb water without disintegrating. They boost soil water availability for plants, reduce evaporation and percolation waste, and improve crop development and yield characteristics (El Bergui et al., 2023). Hydrogels function as "miniature water reservoirs" around plant roots. They can collect natural and provided water from 400 to 1500 times their own weight and slowly release it under water-stress circumstances via the root capillary suction mechanism (Patra et al., 2022).

Lettuce is perceived as healthy food by consumers. The nutritional qualities are mostly ascribed to the crop's provision of antioxidant chemicals, such as vitamin C, polyphenols, and vitamin E (Medina-Lozano et al., 2020). It is a rich source of bioactive chemicals with a wide

range of biological activity, including antioxidant, anti-inflammatory, anticancer, antibacterial, cholesterol-lowering, and anti-diabetic properties, as well as an excellent source of fiber, iron, and folate (Mohamed et al., 2021).
One of the most important indications of the nutritional quality of fruits and vegetables is vitamin content, particularly vitamin C. Its beneficial properties are related to its role in various processes in the human body, such as collagen formation, cholesterol reduction, inorganic iron absorption, and, most importantly, immune system enhancement due to its antioxidant activity (Medina-Lozano et al., 2021). Vitamin C is a required vitamin for humans, unlike many other animals, because we are unable to synthesize it due to mutations in the gene coding for the last step enzyme in the biosynthesis pathway. It is necessary for regular cell metabolism and also plays a vital role in immunological responses, owing to its antioxidant action (Medina-Lozano et al., 2020).
The presence of phenolic chemicals in lettuce is regarded to be the fundamental cause for its high antioxidant activity. They are formed in response to environmental stress and other stressors such as UV, radiation, injuries, and processing procedures including postharvest treatment (Materska et al., 2018). Recent studies (Zhang et al., 2023; Hernández-Adasme et al., 2023; Utami & Damayanti, 2023) revealed antioxidant activity in lettuce leaves. Furthermore, lettuce's preventive benefit can be attributed in part to its high antioxidant content, which includes antioxidative enzymes, flavonoid, ascorbate, carotenoid, and tocopherol (Nikzad & Parastar, 2021). The amount of phenolic substances (most notably flavonols, caffeic acids and their derivatives, carotenoids, and vitamins C and E) and antioxidant activity are also noted in lettuce and they are heavily impacted by genetic variables, environmental circumstances, and production systems, among other things (Negrao et al., 2021).
Therefore, this study aimed to analyze the bioactive compounds such as total phenolic content, vitamin C and the antioxidant activity of *Lactuca sativa* under drought stress conditions, using hydrogels based on different concentrations of montmorillonite.

MATERIALS AND METHODS

1. Polymeric Material

The hydrogels used in this experiment were obtained from The National Institute for Laser, Plasma and Radiation Physics, Măgurele, using the electron beam radiation method and potassium persulfate as activator. Four different compositions of hydrogels based on acrylic acid, polyethylene oxide, sodium alginate and montmorillonite were tested. In this experiment two methods of administration of the hydrogels were tested, namely granular form (VG) and beads (VB) (Figure 1, Table 1). Each type of hydrogel was applied in 10 repetitions (5 for drought conditions and 5 for irrigated conditions) for each composition, the weight of 1 bead being of 0.2 g, weight that was kept also in the case of granules. The hydrogels were buried in 50 g soil and 100 ml water was added for each sample to assure the swelling of the polymeric material.

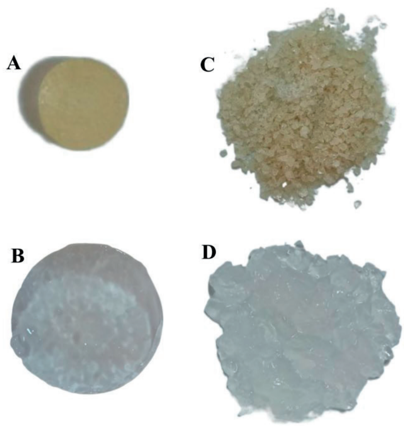

Figure 1. Hydrogel sample:
A. Hydrogel bead; B. Hydrogel bead swollen in water; C. Hydrogel granules; D. Hydrogel granules swollen in water

Table 1. Sample codification used in this study

Code	Type of hydrogel		Montmorillonite concentration (%)
	Bead	Granular	
V1	V1B	V1G	0
V2	V2B	V2G	0.25
V3	V3B	V3G	0.5
V4	V4B	V4G	1
M	Control sample with no hydrogel and drought conditions		

2. Plant Materials and Growing Conditions
The lettuce (*Lactuca sativa*) seedlings placed in alveolar tray, were acquired from SCDL Buzău and used in the experimental tests as follows: the seedlings were successively transplanted in cups, pots and after a period of 35 days they were planted in a greenhouse (Figure 2) where 2 different conditions were applied, one simulating the drought stress conditions (without irrigation) and the other with regularly irrigated plants.

A B C D

Fig. 2 Lettuce sample (*Lactuca sativa*) on different stages of growth:
A. Lettuce seedling; B. Lettuce transplanted in cups; C. Lettuce sample after 21 days (in pot); D. Lettuce sample after 25 days (in greenhouse)

3. Biochemical analysis
Biochemical determinations were further conducted, in order to determine the content in vitamin C, total phenolics and antioxidant activity.
Vitamin C. For the total vitamin C content determination, 10 g of fresh lettuce leaves were grounded and mixed with 20 ml of 2% oxalic acid. The obtained mixture was brought to 100 ml and let to extract for 15 minutes. Further, 2 ml of the filtered extract was mixed with 1 ml of 2% oxalic acid, 5 ml buffer solution, 2 ml of indophenol (2,6-Dichlorophenol Indophenol) and 20 ml xylene. The obtained solution was then centrifuged for 20 min at 4°C and 9000 rpm. Sample absorbance was measured at 500 nm and the results were expressed as mg/100g dry weight (DW).
Total phenolic content. The Folin–Ciocalteu method was used to determine total phenolic content. Briefly, 5 g of fresh chopped leaves were extracted in 25 ml ethanol 75% for a period of 48h in the dark. Further, 20 µl of filtered extract was mixed with 1.58 ml distilled water, 100 µl Folin-Ciocalteu reagent and 300 µl of 20% sodium carbonate. The obtained samples were incubated at room temperature for 2h in the dark, and then, the absorbance was measured at 765 nm. The results were expressed as mg GAE (gallic acid equivalents) /100g dry weight (DW).
Antioxidant activity. For antioxidant activity determination, the DPPH method was used. Therefore, 5 g of fresh chopped leaves were extracted in 25 ml ethanol 75% for a period of 48h in the dark. Then, 0.05 ml of filtered sample was mixed with 1.950 ml DPPH solution. After incubation at room temperature for 30 min in the dark, the absorbance of the samples was measured at 515 nm and the results were expressed as mg QE (Quercetin Equivalents)/100 g dry weight (DW).

RESULTS AND DISCUSSIONS

The biochemical analyses were performed initially and after 3, 20 and 40 days from the initiation of the simulated drought process.

Vitamin C content
The vitamin C content during the drought induced stress after 3 days showed that the samples containing higher concentrations of montmorillonite are proven to be more beneficial as seen in Figures 3 and 4. After 20 days of drought the progressive decrease of vitamin C content can be noticed, the samples that contained no montmorillonite having the lowest amount of ascorbic acid concentration, similar to the Control sample. After 40 days under drought conditions, the vitamin C content decreased considerably for each sample showing a noticeable difference between the Control and the plants grown in the presence of hydrogels (either bead or granular form hydrogels). Between the 2 forms of administration, the bead form showed better results for the vitamin C content (Figures 5 and 6).

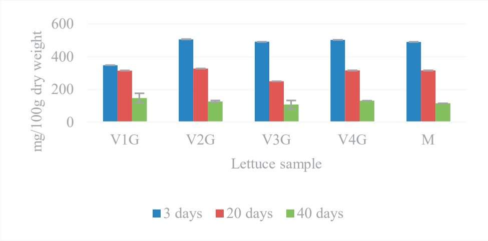

Figure 3. The evolution of vitamin C accumulation under induced drought for the granular hydrogels

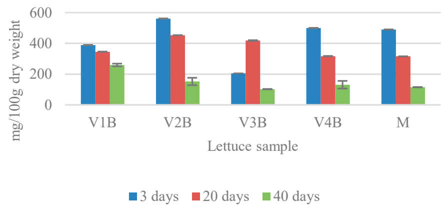

Figure 4. The evolution of vitamin C accumulation under induced drought for the bead hydrogels

The ascorbic acid content during the drought conditions showed higher values for both forms of administration compared to the usual content range of vitamin C in *Lactuca sativa*. In a review conducted by Kim et al. (2016) on different lettuce types, the range of ascorbic acid oscillated between 8.40 and 390 mg/100 g DW. The result of our study showed that vitamin C values remained in the range, even after the 40 days of induced drought stress, the values oscillating between 107 and 259 mg/ 100 g DW.

After 40 days, the vitamin C content was slightly higher for the plants under irrigated conditions as showed in Figure 5. Compared to the Control, most samples showed higher content of ascorbic acid for both forms of hydrogel administration and irrigating conditions.

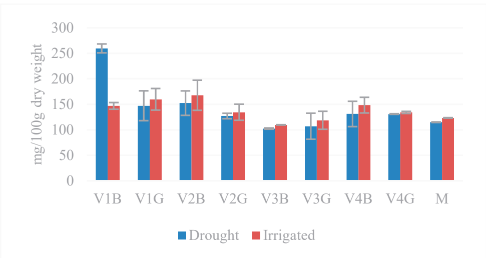

Figure 5. Vitamin C content after 40 days of induced drought stress and regular irrigation

Antioxidant activity

The antioxidant activity under drought conditions as showed in Figures 6 and 7, proved to be higher as the stress induced by the drought increases over time. Therefore, the longer the period of induced drought stress, the higher the antioxidant activity, as reported also by other authors (Liava et al., 2023; Pan et al., 2023).

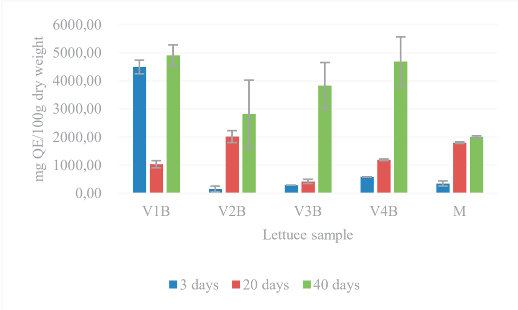

Figure 6. Evolution of the antioxidant activity under induced drought stress for bead hydrogels

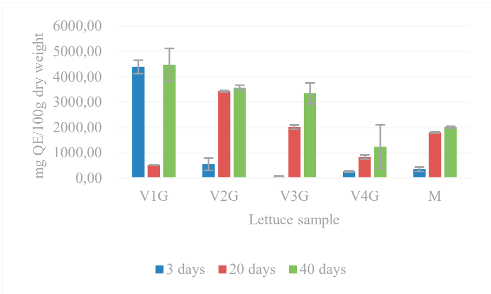

Figure 7. Evolution of the antioxidant activity under induced drought stress for granular hydrogels

The plants that were exposed to drought conditions presented significantly increased antioxidant activity for both forms of administration of hydrogels compared to the Control sample, most likely as a metabolic response to water stress, as it is also described in literature by various authors for different plant species (Talbi et al., 2020).

Samples in the presence of hydrogels with lower concentrations of montmorillonite (V2-V3) on either form of administration proved to have considerable effect on reducing the stress induced by the severe drought conditions, slightly similar to the Control plants (Figure 8).

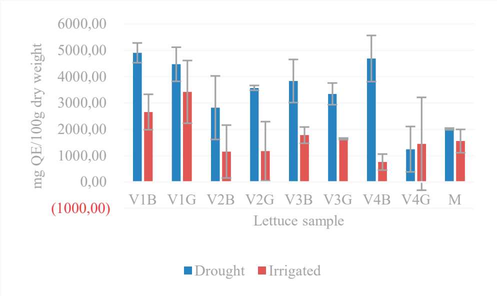

Figure 8. The antioxidant activity after 40 days of induced drought stress and regular irrigation

Total polyphenolic content

The total polyphenolic content showed similar results as the ones obtained for antioxidant activity under the drought induced stress. Higher values were observed as the drought period increased (Figures 9 and 10).

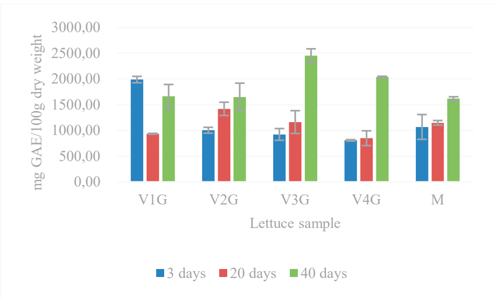

Figure 9. Evolution of the total polyphenol content under induced drought stress for granular hydrogels

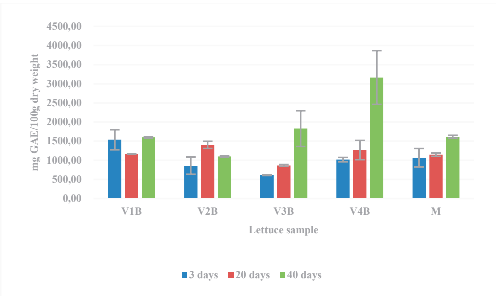

Figure 10. Evolution of the total polyphenol content under induced drought stress for bead hydrogels

In a study conducted by Liu et al. (2007) on different types of *Lactuca sativa*, they obtained the total phenolic content in a range between 1840-5640 mg GAE/100 g dry weight. Results of our study showed lower content in total phenolics, the values oscillating between 613 (V3B) and 3164 (V4B) mg GAE/100 g DW, which increased over time during the drought induced stress.

After 40 days, the total polyphenolic content proved to be higher for the plants that were exposed to the drought conditions, similarly to the results regarding the antioxidant activity. Samples containing hydrogels with higher concentrations of montmorillonite (V3-V4) presented better results regarding the total polyphenols accumulation during both drought stress as well as under irrigated conditions (Figure 11).

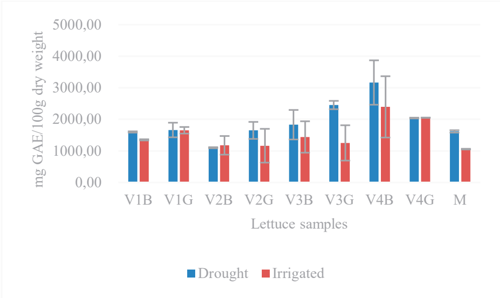

Figure 11. The polyphenols content after 40 days of induced drought stress and regular watering conditions

Therefore, analysing the obtained results, a selection of the form of administration of the hydrogel (bead or granules) can be made, based on the correlation that exists between the water stress and the antioxidant activity of the plant, namely the longer the period of exposure to water stress, the higher the antioxidant activity. Thus, according to the results of this study (Figure 12), we can conclude that for all the variants of tested hydrogels and both methods of administration (V1, V2, V3, V4), the granular form proved to alleviate the water stress induced by the extensive period of drought, compared to the bead administration form of the same hydrogels and even compared to the Control for V4 samples.

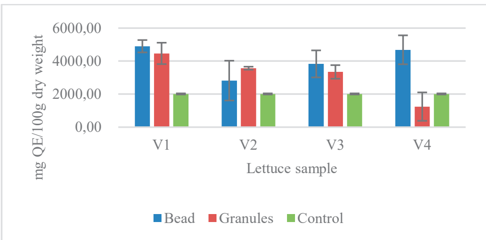

Figure 12. The antioxidant activity of Lactuca sativa after 40 days of drought stress

CONCLUSIONS

The results showed that the usage of hydrogels containing montmorillonite proved to be beneficial for water management regarding long periods of drought. Between the two forms of administration, the granular form of hydrogels was the best in alleviating the water stress induced by the extensive period with no irrigation compared to the bead administration form of the same hydrogels. The samples containing higher amount of montmorillonite proved to have considerable effect on reducing the stress induced by the severe drought conditions, slightly similar to the control plants while also presenting better results regarding the total polyphenols accumulation during both drought stress as well as under irrigated conditions. The ascorbic acid content was slightly higher for the regularly watered plants. However, the obtained results showed that the values of the studied parameters at the end of the testing period were not significantly different between the two cultures; therefore, the tested hydrogels present great potential to be used for water management in agriculture. Furthermore, higher concentrations of montmorillonite in the hydrogel composition showed increased tolerance to drought stress compared to no or lower concentrations as well as the Control plants.

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RESULTS REGARDING THE 24-HOUR COMPOSTS (OKLIN) COMPOSITION AND THEIR USE AS FERTILISER

Adrian George PETICILĂ, Roxana Maria MADJAR, Elena Maria DRĂGHICI, Florin STĂNICĂ, Adrian ASĂNICĂ, Raluca CIUBOTĂRĂŞU, Ana Cornelia BUTCARU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: anabutcaru@gmail.com

Abstract

The paper aims to present some of the results of the project "Research on the use of composts obtained from food waste", where more compost variants obtained with OKLIN series composters were analyzed and tested on two horticultural species (germination and total growth). According to the specifications, OKLIN composters are designed to produce compost 24 hours after the organic residues are introduced. Compost obtained from different sources (grounds of coffee, vegetables (40%) and fruits (60%), vegetables (40%), fruit (50%), and fish (10%), food and vegetable waste (100%), etc) were analyzed regarding microbiota (bacteria, fungi, and respiration coefficient) and agrochemical parameters. Several ratio variants were tested on parsley (Petroselinum crispum) and Luffa, monitoring germination rate and total growth. All the compost variants, in general, presented beneficial and neutral bacteria and fungi with high potential as fertilizer. 10% and sometimes 20% were the most favorable compost ratios as fertilizer for the horticultural plants tested.

Key words*: bacteria, fungi, composter.*

INTRODUCTION

Food waste management, along with other categories of waste, has become an increasingly current problem in many countries caused by the increase in population and the need and consumption of food (Chen, 2016). Following studies by Chen et al. (2020), 1.13 million tons of food waste were thrown away worldwide daily. At the level of the European Union, 89 million tons of food waste are thrown away annually, and in Romania, 5 million tons (Romania - Insider, 2020).

These figures are continuously increasing both due to the growth of the population and its purchasing power, an aspect with direct effects on the environment, greenhouse gas emissions, and water pollution (Malamis et al., 2017; Scherhaufer et al., 2018; Wei et al., 2017).

At the level of the European Union, waste management is regulated by Directive 851/2018 of the European Parliament and of the Council amending Directive 98/2008/EC.

Non-hazardous waste management activity in Romania is regulated by Law 181/2020, which will be in force starting February 2021. This regulates, among other things, the conditions for the use of non-hazardous waste for composting, establishes the categories of waste intended for composting and the quality categories of compost, the methods of use of compost, the ways to establish the quality of compost according to European regulations, the obligations of economic operators and the measures to supervise the compost market in Romania.

Regulated activities for food waste treatment are animal feeding (Mo et al., 2018), pyrolysis (Kim et al., 2020), incineration (You et al., 2016), anaerobic/aerobic digestion (Wainaina et al., 2020), and composting (Cesaro et al., 2019). Composting is the most technically, economically, and ecologically viable and reliable of all these activities (Awasthi et al., 2020).

Compost is the result of the process of decomposition of organic matter, called composting.

In 1983, Bertoldi et al., defined composting as a way to obtain a stable product through an oxidative biological transformation similar to what happens in nature. Other authors define composting as a natural decay/decomposition process that changes organic waste into a humus-rich material called compost (Mustin, 2016).

A broader definition is given by Tiquia et al. (2000) in which composting is an effective and safe method by which the mass and volume of organic waste can be reduced (food waste, manure, vegetable residues of agricultural and horticultural crops, the organic fraction of municipal solid waste, etc.) can destroy pathogens and stabilize nutrients and organic matter. Composting occurs naturally and continuously in nature without human intervention; practically everything organic is a potential compost.

Composting occurs in the presence of oxygen, through which organic matter is decomposed by microorganisms, under controlled conditions. During composting, they consume oxygen, releasing considerable amounts of water vapor, heat, and carbon dioxide (Pace et al., 1995).

Often, in recent years, the question arises: Why compost? Here are some arguments:

➢ uses materials that are otherwise useless;

➢ reduce waste;

➢ it results in a safe and effective modification of the soil, by supplying essential nutrients to the plants;

➢ reduce the costs of mulching and fertilization;

➢ it is completely ORGANIC!

➢ it is a way for nature to return to nature.

A quality compost must present certain hydrophysical characteristics (easy release of water, high water retention capacity, high aeration capacity, medium porosity and density, specific electrical conductivity) and chemical (pH, cation exchange capacity, organic carbon, and ash) (Corti et al., 1998.) and depending on them the intended use of the compost is different.

Obtaining quality compost is given by the raw material, as well as the conditions and time in which composting is carried out (Soto-Paz et al., 2019). In this sense, there are several categories of raw materials: urban organic waste (households, restaurants, fast food, grocery stores, food markets, etc.), plant materials (gardening, urban vegetation cleaners, leaves, etc.), garbage from animals, food waste, sludge from wastewater treatment plants, lignocellulosic biomass (agricultural residues, energy crops, forest residues), etc.

Depending on the collection system, food scraps may also contain a high percentage of inert materials such as glass or plastic, which can affect the quality of the compost. Also, food waste has a high moisture content, inadequate C/N ratio, and low aeration rate, all of which lead to an improper composting process with foul-smelling emissions and a negative effect on the environment and obtaining compost of poor quality.

These shortcomings can be countered by using co-composting or bulking agents, such as green leaves, straw, hay, manure, sawdust, etc. (Oviedo-Ocana et al., 2019). The ratio of the components mixed to form the compost and their aeration in the composting process are considered operational strategies to minimize the lack of all materials and shorten the composting process (Ge et al., 2015). The mixing ratio or C/N ratio of the components participating in food waste composting was set within 15-25/30. A ratio of less than 15/30 may reduce the need to add co-components but foul-smelling substances such as ammonia may be released. (Kumar et al., 2010). The performance of the composting process can be increased in terms of nutrient losses and gas emissions over time by the simple process of aeration (Kalamdhad & Kazmi, 2009; Waqas et al., 2018).

Aeration is given by the rotation frequency so that, too excessive but also too low, it influences the decomposition of organic matter through variations in the humidity and temperature of the compost, ultimately resulting in nutrient losses (Kalamdhad & Kazmi, 2009). Fan et al. (2018) showed that an efficient composting of vegetable food waste can be done together with garden waste (grasses, leaves) in proportion of 40% to which dry leaves and rice bran can be added with a role in increasing the microbial community. In this case, the time was about 60 days with a rotation frequency of 3 times a week.

If the optimal composting parameters are not respected, unpleasant odors may appear. They have a negative impact on the environment, leading to the closure of composting facilities (Colon et al., 2012).

The primary source of these odors is volatile organic compounds, the most frequent being terpenes, aliphatic carbons, aromatic hydrocarbons, ketones, and esters (Zhang et al., 2016). Other authors (Komilis et al., 2004) established that the most abundant groups of volatile organic compounds in food waste were sulfides, acids, and alcohols. Their presence may result from reactions that occur during the food cooking process, due to the small amounts of pesticides present on raw vegetables or as a result of atmospheric deposition (Cerda et al., 2018). A low C/N ratio leads to the release of ammonia (NH_3), which is derived from nitrogen compounds and the most critical pollutant resulting from food waste composting (Zang et al., 2016). Pagans et al. (2006) point out that ammonia release is favored by a high pH (alkaline) and a high compost pile temperature. In the case of protein-rich food waste, odors derived from sulfur (dimethyl sulfide, dimethyl disulfide, and methyl mercaptan) can develop (Cerda et al., 2018).

Compliance with the composting parameters, along with the incorporation of gas treatment units in the composting facilities, as well as biofiltration, can reduce the emission of these gases, thus being able to locate these composting facilities near inhabited areas (Cerda et al., 2018).

Composting can be done by several methods depending on the type of food waste, its quantity, the place where the composting is done, and the desired time for obtaining the compost.

Bulk composting (piles of various lengths and shapes) consists of placing food scraps in narrow piles that are aerated by mechanical turning. The frequency of these returns is given by the composition of the waste and the transformation phase. Composting mass aeration can also be forced with pressurized air through bottom suction (negative pressure), bottom blowing (positive pressure) (Feinstein et al., 1983), and alternate ventilation.

Composting in compost bins or containers has applicability inside buildings, containers, and ships. In-vessel composting systems limit the amount of waste used, being able to shorten composting time by monitoring and controlling the entire process. This category includes continuous vertical reactors (the height of food waste can reach 9 m) and horizontal reactors (the height of food waste does not exceed 2-3 m). Of the two, the horizontal one is easier to handle. (Haug & Haug, 1993). The rotating compost bin shortens the composting process to turn food waste into compost takes approx. 4-7 days (Chen, 2016).

The technical method presented in this study involves the use of equipment that produces compost from food waste: Oklin GG-02 composter.

The advantages were:

- Eliminates the need for long-term storage of food waste, as we no longer need specially arranged spaces, thus determining an alignment, without effort, with the legal provisions in force regarding food waste;
- Substantially reduces the costs related to waste collection. Practically, food scraps are removed from the mass of waste that must be given to specialized companies for collection, the collection can be done at longer intervals and in much more advantageous cost conditions.
- Allows for maintaining a high level of hygiene. Odors, microbes, insects, or rodents will no longer appear due to stored food waste, nor will it be necessary to sanitize the spaces where such waste is kept permanently.
- Generates more time for productive activities. Implementing complicated sanitation and waste handling policies and monitoring how the staff applies these policies is no longer necessary.

The paper aims to present some of the results of the project ”Research on the use of composts obtained from food waste”, where more compost variants obtained with OKLIN series composters were analyzed and tested on two horticultural species (germination and total growth).

MATERIALS AND METHODS

The project involved obtaining compost from five different sources: coffee grounds, ground coffee (CAF), food waste (USAMV, CBA, CBV, SBA, CTG, RC), and plant residues (E). In the first stage, the compost variants were analyzed from a mineral, organic, and microbiological point of view.

Compost obtained from different sources (grounds of coffee, vegetables (40%) and fruits (60%), vegetables (40%), fruit (50%), and fish (10%), food and vegetable waste (100%) were analyzed regarding microbiota (bacteria, fungi, and respiration coefficient) and agrochemical parameters.

Table 1. Testing substrate variants (%)

Compost / Variants	S1 vegetables (40%) and fruits (60%)	S1 vegetables (40%) and fruits (60%)	Coffee	Coffee	S2 vegetables (40%), fruits (50%), and fish (10%)	S2 vegetables (40%), fruits (50%), and fish (10%)	S3 vegetables (40%), fruits (50%), and fish (10%)	S3 vegetables (40%), fruits (50%), and fish (10%)	CTG	CTG	Peat	Per lite
V0	-	-	-	-	-	-	-	-	-	-	80	20
V1	10	-	-	-	-	-	-	-	-	-	80	10
V2	-	20	-	-	-	-	-	-	-	-	70	10
V3	-	-	10	-	-	-	-	-	-	-	80	10
V4	-	-	-	20	-	-	-	-	-	-	70	10
V5	-	-	-	-	10	-	-	-	-	-	80	10
V6	-	-	-	-	-	20	-	-	-	-	70	10
V7	-	-	-	-	-	-	10	-	-	-	80	10
V8	-	-	-	-	-	-	-	20	-	-	70	10
V9	-	-	-	-	-	-	-	-	10	-	80	10
V10	-	-	-	-	-	-	-	-	-	20	70	10
V11	1	-	-	-	-	-	-	-	-	-	89	10
V12	-	5	-	-	-	-	-	-	-	-	85	10
V13	-	-	1	-	-	-	-		-	-	89	10
V14	-	-	-	5	-	-	-	-	-	-	85	10
V15	-	-	-	-	1	-	-	-	-	-	89	10
V16	-	-	-	-	-	5	-	-	-	-	85	10
V17	-	-	-	-	-	-	1	-	-	-	89	10
V18	-	-	-	-	-	-	-	5		-	85	10
V19	-	-	-	-	-	-	-	-	1	-	89	10
V20	-	-	-	-	-	-	-	-	-	5	85	10

Several ratio variants were tested on parsley (*Petroselinum crispum*) and Luffa, monitoring germination rate and total growth (Table 1).
For the descriptive statistics of the data, Microsoft Excel 2016 and IBM SPSS v. 28.0.1.1 with a significance level of p = 0.05 were used.

RESULTS AND DISCUSSIONS

The following results were obtained for the quantitative microbiological analysis of several variants of compost and substrate.

Bacteria

The compost variants showed values between 2020 viable cells/g dry soil (CAF) and 99,012,000 viable cells/g dry soil (coffee). The substrates, however, showed values between 473,955,000 viable cells/g dry soil (V6) and 883,448,000 viable cells/g dry soil (V2).
V3 presented at the time of analysis 492,780,000 viable cells/g dry soil and V9 716,892,000 viable cells/g dry soil (Figure 1).
The experimental variant V3 presented among the identified taxa: *Arthrobacter globiformis, Bacillus cereus, Bacillus cereus* var. *mycoides, Bacillus megaterium, Bacillus mesentericus, Pseudomonas fluorescens, Pseudomonas* sp., *Actinomycetes Series Albus* known for their positive influence on plant growth.
In the experimental variant V9, the species *Arthrobacter globiforms, Bacilllus circulans, Bacillus megaterium, Bacillus polymixa, Bacillus subtilis, Paenibacillus* sp., *Pseudomonas fluorescens, Pseudomonas* sp., *Actinomycetes Series Fuscus* predominated.

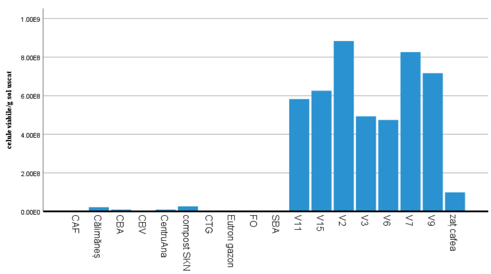

Figure 1. Quantitative determinations of bacteria (viable cells/g dry soil)

Fungi

The compost variants presented values between 2 cfu/g dry soil (E) and 103,281 cfu/g dry soil (coffee). The substrates showed values between 12,593 cfu/g dry soil (V6) and 52,640 cfu/g dry soil (V9). V3 was presented at the time of analysis in 27,088 cfu/g dry soil. (Figure 2).
The V3 variant presented *Trichoderma harzianum* and *Actinomucor elegans* among the species and the V9 variant *Verticillium tenerum*, *Rhizpus stolonifera*, and *Trichoderma harzianum*.

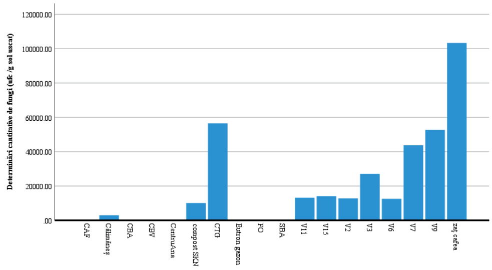

Figure 2. Quantitative determinations of fungi (cfu/g dry soil)

Compost and substrate macro and microelements

Table 2 presents minerals and contaminants for the compost and substrate variants.
For the nitrogen, values ranged between 1.69% (CBV) and 2.64% (CAF), and for substrate, between 1.04% (V3) and 1.17% (V2). Phosphorus ranged between 0.206 (SBA) and 0.284 (CTG) at compost and 0.065% (V11) and 0.290% (V6). Potassium content in compost was between 0.91% (coffee ground) and 3.53 (CAF), respectively, 0.13% (V15) and 0.43 (V6). Calcium, sodium, magnesium, zinc, copper, manganese, iron, lead, cadmium, cobalt, and chromium were analyzed also (Table 2).

The influence of experimental variants on germination and growth in parsley

Germination

The parsley germination rate, determined 7 days after sowing, is presented in Figure 3.
The experimental variant PV10 (20% CTG) presented the best germination rate (97%), followed by PV6 (20% S2) and PV3 (10% coffee) with 87%, PV7 (10% S3) and PV4 (20% coffee) with 77%. The variants PV9 (10% CTG) and PV5 (10% S2) showed a germination rate similar to the control variant (70%).

Table 2. Compost and substrate macro and micro elements analysis

Variant	N	P	K	Ca	Na	Mg	Zn	Cu	Mn	Fe	Pb	Ni	Cd	Co	Cr
Compost	%	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Coffee ground	2.21	0.230	0.91	0.85	-	0.15	25.2	13	15.5	23.1	-	-	-	-	-
CBA	1.86	0.229	1.35	0.29	0.28	0.096	9.8	3.5	10.6	18	nd	nd	nd	0.3	1.1
CBV	1.69	0.259	1.63	0.29	0.17	0.111	15.7	4.8	13.5	49	nd	2.2	nd	0.6	9.7
SBA	1.11	0.206	3.34	0.13	0.18	0.093	11.0	6.6	18.4	64	nd	0.6	nd	0.2	5.0
CAF	2.64	0.223	3.53	0.79	0.16	0.164	6.2	10.1	12.6	25	nd	nd	nd	0.4	17.9
CTG	2.35	0.284	1.27	1.27	0.50	0.100	21.4	4.8	15.8	68	nd	nd	nd	0.5	10.3
RC	1.74	0.228	1.30	0.78	0.257	0.095	44.1	6.72	19.9	152	1.3	1.00	nd	0.27	6.4
E	2.59	0.234	2.22	0.253	0.048	0.173	67.7	10.8	30.2	817	0.90	1.5	nd	0.05	29.5
Substrate															
V2	1.17	0.233	0.24	1.05	0.131	0.228	26	3.7	16	909	nd	nd	nd	nd	2.3
V6	1.08	0.290	0.43	0.74	0.113	0.183	23	2.4	15	841	nd	nd	nd	nd	5.9
V11	0.92	0.065	0.17	1.30	nd	0.254	11	2.7	14	639	nd	nd	nd	nd	nd
V15	0.83	0.069	0.13	1.30	nd	0.247	9.7	2.5	16	1228	nd	nd	nd	nd	0.5
V3	1.04	0.170	0.314	0.227	0.124	0.213	16.1	3.6	21	852	0.885	nd	nd	nd	nd
V7	1.11	0.200	0.264	0.247	0.148	0.209	14.8	2.7	21	733	0.614	nd	nd	nd	1.18
V9	1.07	0.086	0.186	1.150	0.157	0.229	14.1	3.1	21	657	0.549	nd	nd	nd	nd

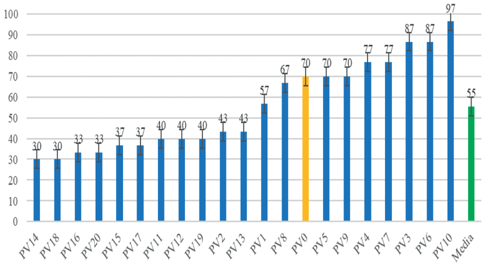

Figure 3. Germination rate (%) in parsley at 7 days from the time of sowing

The lowest values were recorded for the experimental variants PV14 (5% coffee) and PV18 (5% S3) with 30%, PV16 (5% S2) and PV20 (5% CTG) with 33% respectively, PV15 (1% S2) and PV17 (1% S3) with 37%.

Plant growths

The variants with the highest growth (similar values) were PV0 (control), PV1 (10% S1), PV17 (1% S3), PV3 (10% coffee) and PV9 (10% CTG).

Variants V2 (20% S1), V4 (20% coffee), V6 (20% S2), and V8 (20% S3) presented the lowest values in plant growth at several 14 days from the time of sowing (Figure 4).

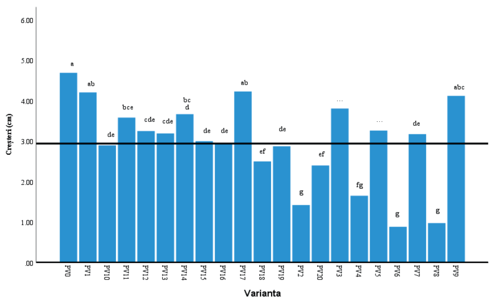

Figure 4. Parsley plant growth at 14 days from sowing

Centralizing the two parameters (Table 3), germination rate and plant growth, in the *Petroselinum crispum* species, the variants PV3 (10% coffee) and PV9 (10% CTG) obtained maximum results in both parameters.

The lowest values were obtained in composts with lower concentrations (1% or 5%) for the germination rate. In plant growth, lower values were obtained with four types of composts with concentrations of 20%.

Table 3. Centralization of maximum and minimum values for the two analyzed parameters

Maximum		Minimum	
Germination rate	**Growth**	**Germination rate**	**Growth**
PV10	PV0, PV1, PV17, **PV3, PV9**	PV14, PV18	V2
PV6		PV16, PV20	V4
PV7, PV4		PV15, PV17	V6
PV9, PV5, PV0			V8

The influence of experimental variants on germination and growth in *Luffa cilindrica* plants

Germination

The loofah germination rate, calculated 33 days after sowing, is presented in Figure 5. The experimental variant LV6 (20% S2) presented the best value (97%) being, followed by LV8 (20% S3) with 93%. Very close and with similar values are the following variants: LV10 (20% CTG), LV7 (10% S3), LV4 (20% coffee), LV2 (20% S1), LV9 (10% CTG), LV5 (10 % S2), and LV3 (10% coffee).

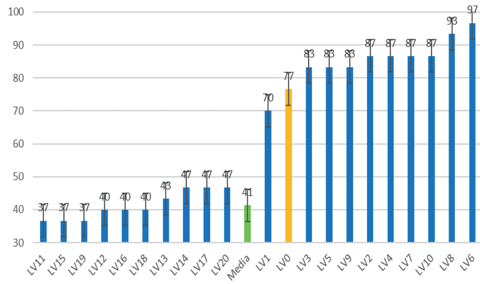

Figure 5. Germination rate (%) *Luffa cilindrica*

The lowest values were recorded for the experimental variants LV11 (1% S1), LV15 (1% S2), LV19 (1% CTG) with 37%, LV12 (5% S1), LV16 (5% S2) and LV18 (5% S3) with 40%.

Plant growths

33 days after sowing, in the experimental variants LV0 - LV10, the growth varied between 11.25% (LV6 - 10% S2) and 28.98% (LV3 - 10% coffee) and in the experimental variants LV11 - LV20 between 13% (LV15 - 5% coffee) and 23.92% (LV12 - 1% S1) (Figure 6).

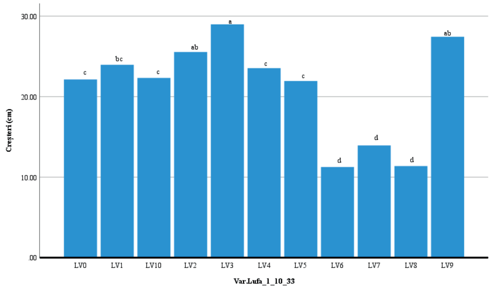

Figure 6. Growth of loofah plants 33 days after sowing

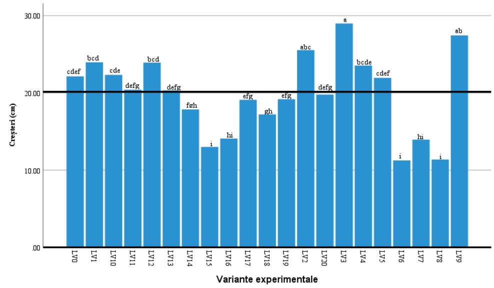

Figure 8. Growth of loofah plants 33 days after sowing

The highest values were recorded for the experimental variants LV3 (10% coffee), LV9 (10% CTG) and LV2 (20% S1).
The lowest values were recorded for the experimental variants LV6 (20% S2), LV8 (20% S3), and LV7 (10% S3).
Thirty-three days after sowing, the experimental variants LV11 - LV20 had the plant growth values shown in Figure 7. The best results were obtained by the variant LV12 (5% S1), and the smallest variants were LV15 (1% S2) and LV16 (5% S2).

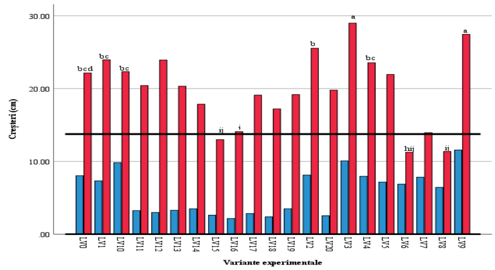

Figure 9. Growth of loofah plants (LV0-LV20) July (blue) and August (red)

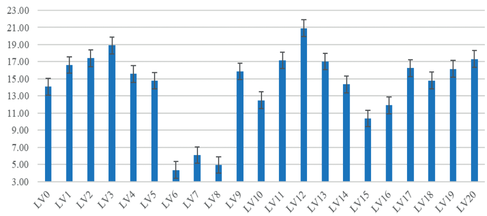

Figure 10. The growth rate between the two measurements

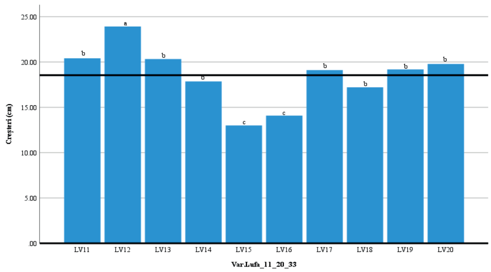

Figure 7. Loofah plant growth 33 days after sowing (LV11-LV20)

Analyzing all the experimental variants 33 days after sowing, we have the distribution shown in Figure 8. The best growth was given by the experimental variant LV3 (10% coffee), similar to LV9 (10% CTG) and LV2 (20% S1). The smallest values were present in variants LV6 (20% S2), LV8 (20% S3), and LV15 (1% S2).
Analyzing the growth of the plants comparatively in the two moments when the measurements were made (July and August) (Figure 9), differences in the increase in growth are noted (Figure 10). The most significant increase was in the LV12 variant (5% S1), followed by LV3, LV2, and LV20.

Table 4 shows the variants with the best or lowest response for loofah, which are centralized.

Table 4. Maximum and minimum values for the two analyzed parameters for loofah

Maximum		**Minimum**	
Germination rate	**Growth**	**Germination rate**	**Growth**
LV6	**LV3, LV9**	LV11, **LV15,** LV19	LV6, LV8 **LV15**
LV8	**LV2**	LV12, LV16, LV18	
LV10, LV7, LV4, **LV2, LV9,** LV5, **LV3**			

CONCLUSIONS

All the compost variants, in general, presented beneficial and neutral bacteria and fungi with high potential as fertilizer. 10% and sometimes 20% were the most favorable compost ratios as fertilizer for the horticultural plants tested.

Analyzing the two species, we notice that the compost variants that gave the best values are present in both analyses, and V3 (10% coffee) and V9 (10% CTG) had the best yield.

The lowest values were obtained at 1% and 5% concentrations for different compost variants. Also, for concentrations of 20% at V6 (20% S2) and V8 (20% S3).

ACKNOWLEDGEMENTS

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THE QUANTITATIVE ESTIMATION OF SOME BIOCHEMICAL COMPOUNDS WITH ANTIOXIDANT PROPERTIES IN THE FRUITS OF THREE CULTIVARS OF *ELAEAGNUS UMBELLATA* THUNB. INTRODUCED IN THE REPUBLIC OF MOLDOVA

Ion ROȘCA, Elizaveta ONICA, Aliona GLIJIN, Alina CUTCOVSCI-MUȘTUC, Vitalie MÎȚU

"Alexandru Ciubotaru" National Botanical Garden (Institute),
18 Padurii Str., Chisinau, Republic of Moldova

Corresponding author emails: amustuc@gmail.com, alinacutcovschi@mail.ru

Abstract

The article includes the results of the phytochemical evaluation in frozen and dried fruits of three taxa of Elaeagnus umbellata Thunb. ('Amoroso', 'Fortunella' and 'Sweet ˈnˈ Sour'). The analysis of ascorbic acid revealed values between 85.97 and 99.69 mg/100 g in frozen fruits and 127.62 - 135.34 mg/100 g in dried fruits. Irrespective of the type of fruit preservation, the maximum amount of vitamin C was detected in the cultivar 'Amoroso'. The minimum amount of tannins was found to be characteristic of the cultivar 'Sweet ˈnˈ Sour' in both frozen (1.25%) and dried (1.88%) fruits. The total phenolic content recorded values from 1037.61 mg GAE/100 g d.m. to 1183.64 mg GAE/100 g d.m. in frozen fruits and from 1121.14 mg GAE/100 g d.m. to 1260.34 mg GAE/100 g d.m. in dried fruits. The comparative analysis of the obtained data allowed us to conclude that the fruits of analysed taxa are a promising source of natural antioxidants, which provide them with powerful antioxidant properties, and drying proved to be a more effective method of preserving the fruits than freezing, maintaining a higher amount of active principles.

Key words: *Elaeagnus umbellata, autumn olive, biochemical parameters, ascorbic acid, tannins, phenolic compounds.*

INTRODUCTION

Elaeagnus umbellata (Thunb.), commonly known as autumn olive, Japanese silverberry or spreading oleaster, is native to Southern Europe and Central Asia (Dirr, 1998).

The nutritional and therapeutic value of autumn olive fruits is due to their composition and nutraceutical diversity. Thus, the fruits of *Elaeagnus umbellata* are a rich source of vitamins (particularly vitamin A, C and E), minerals (phosphorus, potassium, calcium, magnesium and iron), flavonoids, essential fatty acids, alkaloids, terpenoids, saponins and other bioactive compounds (Matthews, 2001; Wu, Hu and Yang, 2011; Fordham et al., 2001; Fordham et al., 2002; Bhuvaneswari and Nagini, 2005; Perveen et al., 2015; Patel, 2015; Khattak, 2012).

Besides, the fruits contain the vitamins B_1, B_2, B_3, B_6, biotin, folic acid and vitamin K (Aziz et al., 2015). Carotenoids, such as lycopene, α-cryptoxanthin, β-cryptoxanthin, β-carotene, lutein, phytoene, phytofluene, etc., are also valuable compounds. Autumn olive red fruits contain 17 times more lycopene than fresh tomatoes (Fordham et al., 2001).

Due to the antioxidant properties of biologically active substances from fruits, seeds and leaves, the species *Elaeagnus umbellata* is attributed phytotherapeutic properties that are beneficial in the treatment of diseases involving oxidative stress: rheumatoid arthritis, fever, asthma (Niknam et al., 2016), type 2 diabetes (Nazir et al., 2018), cardiovascular diseases (Qayyum et al., 2019) and breast cancer (Jabeen et al., 2020).

Several studies were focused on the analysis of autumn olive as a rich source of lycopene, which is considered an important phytonutrient and is believed to protect against heart attack (Kohlmeier et al., 1997) and various forms of cancer (Clinton, 1998), including prostate cancer (Giovannucci et al., 1995). In recent years, the *E. umbellata* has also been researched as a species with antidiabetic potential (Nazir et al., 2018; Spínola et al., 2019), with an important role in inhibiting the progression of diabetes (Nazir et al., 2021). The potential of autumn olive fruits as an anti-inflammatory, anti-nociceptive (Ahmad et al., 2009; Uddin and Rauf, 2012; Hamidpour et al., 2016; Özen et al., 2017) and antiproliferative agent (Wang, Bowman and Ding, 2007; Özen et al., 2017) has also been mentioned.

The therapeutic value of *Elaeagnus umbellata* against heart diseases and other health problems may be due to the presence of a large amount of oil in the fruits. Vegetable oil and phytosterols are known to have anticoagulant properties, which are recommended for lowering blood cholesterol and treating angina (Fordham, 2001). The seeds and flowers of *Elaeagnus umbellata* are used as a remedy in cardiovascular disorders such as hypertension and also as stimulants in coughs and bowel disorders (Chopra, Nayar and Chopra, 1969; Hussain, 2011).

Relevant studies carried out also include those referring to the phytotherapeutic role of the respective species on antibacterial (Sabir et al., 2007), antifungal, insecticidal and phytotoxic activity (Aziz et al., 2015). The investigations carried out by Sabir et al. (2007) demonstrated the antibacterial activity of aqueous extracts of *E. umbellata* fruits in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*.

Taking into consideration the appreciable medicinal and nutritional value of autumn olive, particularly due to the organic substances involved in redox processes, we set out to quantitatively explore the amount of some biochemical compounds in the fruits of three taxa of *E. umbellata* Thunb.

MATERIALS AND METHODS

The biological material used for research consisted of dried and frozen fruits of three taxa of *Elaeagnus umbellata* Thunb. ('Amoroso', 'Fortunella' and 'Sweet ˈnˈ Sour'), which have been introduced in the collection of "Alexandru Ciubotaru" National Botanical Garden (Institute) in 2018 and began to bear fruit abundantly three years later.

The taxa of *Elaeagnus umbellata* 'Amoroso', 'Fortunella' and 'Sweet ˈnˈ Sour' are melliferous, fruit-bearing, silvicultural and ornamental shrubs. They can tolerate drought and frost. The researched shrubs grow and bear fruit regularly, thriving in sunny places, drained and humus-rich soils, to obtain high productivity and organic, high-quality fruits, rich in bioactive compounds. The three researched taxa of *Elaeagnus umbellata* differ in the colour of the flowers, the number of flowers per flowering stalk, the number of flowering stalks per shoot developed in the previous year, the average weight of fruits, seeds, their size, mesocarp yield and percentage of fruit set. The cultivar 'Fortunella' differs significantly from the other two cultivars in the larger size and weight of fruits and seeds, higher density of flowers and fruits per 20-cm-long shoot, and 'Amoroso' – by the lowest indices of fruit diameter and fruit weight, respectively (Onica et al., 2021). The general aspect of the investigated taxa, in the fruit development stage, is shown in Figure 1.

1 2 3

Figure 1. The general aspect of *Elaeagnus umbellata* Thunb. plants in the fruit development stage
(1 - 'Amoroso', 2 - 'Fortunella', 3 - 'Sweet ˈnˈ Sour')
(Onica, Roșca and Cutcovschi-Muștuc, 2021;
http://www.lubera.co.uk/plants/soft-fruit/superfood-berries/pointilla-sweet-n-sour)

The biochemical analyses were performed at the Institute of Genetics, Physiology and Plant Protection (Republic of Moldova, Chisinau) by using different biochemical methods.

The quantitative determination of vitamin C content. The determination of the ascorbic acid content included spectrophotometric quantification using potassium hexacyanoferrate. In acidic medium, ascorbic acid stoichiometrically reduces potassium hexacyanoferrate (Fe^{+3}) $K_3[Fe(CN)_6]$ (a red salt) to potassium hexacyanoferrate (Fe^{+2}) $K_4[Fe(CN)_6]$ (a yellow salt), which in the presence of ferric ions produces iron (III) hexacyanoferrate (II) ("Berlin blue") $Fe_4[Fe(CN)_6]_3$.

To determine the concentration of vitamin C in the plant extract, the calibration curve was used and the following formula was applied:

$$K = (49.967 \cdot \text{D opt}) - 11.938 \quad (1)$$

where: D opt - absorbance detection at 680 nm.

To calculate the ascorbic acid content in the sample, the following formula was used:

$$C = \frac{K \cdot V}{m} \cdot 100 \quad (2)$$

where: C - ascorbic acid content, µg/100 g biological material; K - concentration of ascorbic acid per 1 ml de extract, calculated according to the calibration curve, µg/ml; V - total volume of the extract, ml; m - weight of the biological sample, g.

The determination of tannins in the researched biological material consisted in their quantification with potassium permanganate (0.1 N), according to the classical titrimetric method (GOST 19885-74) as a result of the process of oxidation of tannins.

The calculation of the percentage of tannin content was done using the formula:

$$C(\%) = \frac{(a - a_1) \cdot 0{,}004157 \cdot V \cdot 100}{V_1 \cdot m} \quad (3)$$

where: a - the quantity of potassium permanganate consumed to oxidize the tannins in the sample; a_1 - the quantity of potassium permanganate consumed to oxidize the tannins in the control (water and indigo carmine); V - the total volume of the sample; V_1 - the volume of the sample used for quantification; m - the dry mass of the sample, g; *0,004157* - the quantity of tannins oxidized by 1 ml of potassium permanganate (0.1 N), g.

Determination of phenolic compounds. The method of determining phenolic compounds in the investigated biological material (Folin-Ciocalteu, 1927, with the modifications proposed by Singleton, Rossi, 1965) was based on the reaction of phenols with the Folin-Ciocalteu reagent, which is reduced in alkaline medium through the interaction with phenolic compounds, thus producing blue complexes. The reaction products were determined spectrophotometrically at a wavelength of 765 nm.

The content of phenolic compounds in one gram of fresh mass, extracted with ethanol (80%) was expressed in terms of gallic acid equivalents (GAE), which ensures the same optical density of the reaction (determined based on the calibration curve). To construct the calibration curve, gallic acid was used as a standard substance, and to calculate the content of phenolic compounds, the following formula was applied:

$$F = \frac{(C \cdot V)}{m \cdot 1000} \quad (4)$$

where: F - the content of phenolic compounds, mg GAE /100 g d.m.; C - concentration of phenolic compounds determined on the basis of the calibration curve, mg GAE/l; V - total volume of the sample; m - the weight of the sample, g; 1000 - coefficient of converting litre to millilitres.

Statistical processing. The research results were analysed using the Microsoft Excel program. The average was calculated for each parameter and the data were expressed as the average of the repetitions.

RESULTS AND DISCUSSIONS

Ascorbic acid. The importance of ascorbic acid lies in the fact that it acts as an enzyme cofactor, contributes to the decomposition of free radicals and is an acceptor / donor in the transport of electrons in plasma membranes and in chloroplasts (Davey et al., 2000). In addition to its main role as an antioxidant and cofactor in redox reactions, recent studies have suggested an important role of ascorbic acid in the activation of epigenetic mechanisms, which control cell differentiation, the dysfunction of which can lead to the development of certain types of cancer (Fenech et al., 2019). Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional

quality factors in various horticultural crops and has several biological functions in the human body (Lee and Kader, 2000).
The quantitative determination of ascorbic acid in the frozen and dried fruits of the three cultivars of *Elaeagnus umbellata* subjected to research revealed values between 85.97 and 99.69 mg/100 g in the frozen fruits and 127.62-135.34 mg/100 g - in the dried ones. In the fruits of both types of preservation, the maximum amount of vitamin C was detected in the cultivar 'Amoroso', and the minimum - in the cultivar 'Sweet 'n' Sour' (Figure 2).
The values recorded in our research are much higher than those obtained by other researchers. Thus, the data obtained by Khattak (2012), in Pakistan, are significantly lower, demonstrating an ascorbic acid content of 27.2 mg/100 g in *Elaeagnus umbellata* fruits. Gamba et al. (2020) quantified 29.12 mg/100 g fresh mass of vitamin C in autumn olive fruits in north-eastern Italy. Ahmad et al. (2005) estimated even lower vitamin C content (14.1-14.3 mg/100 g). These variations could be conditioned not only by the geographical area and climatic conditions, but also by the cultivar and the stage of ripening at the time of harvesting the fruits.
The comparative study on the content of ascorbic acid in our research demonstrated a closer correlation between its amount and the type of fruit storage than the specific characteristics of the taxa. Thus, the difference in the average content of vitamin C between frozen and dried fruits was about 40%, while the difference between its minimum and maximum value in dried fruits was about 6%, and in frozen fruits - a maximum of 16%.

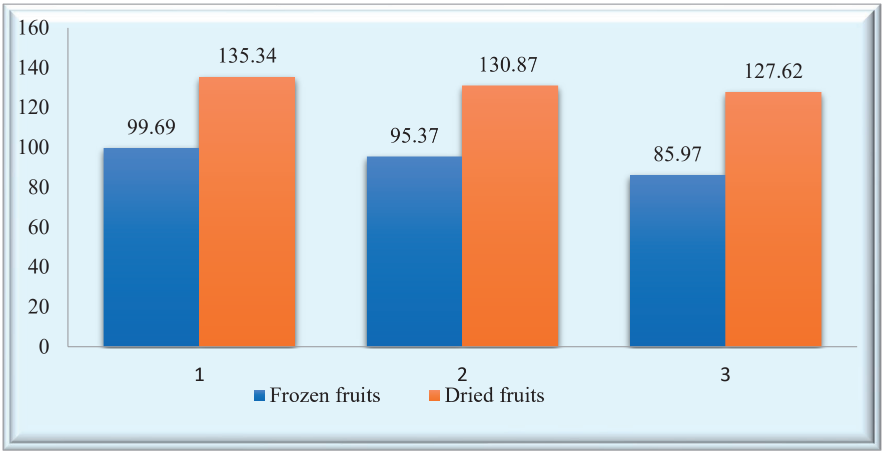

Figure 2. The ascorbic acid content (mg/100 g) in the fruits of different cultivars of *Elaeagnus umbellata* (1 - 'Amoroso'; 2 - 'Fortunella'; 3 - 'Sweet 'n' Sour')

The obtained results allow us to conclude that, in terms of preserving the amount of ascorbic acid, drying is a more advantageous method of storing autumn olive fruits than freezing.

Tannins. Tannins are polyphenols, which have therapeutic properties and act as antioxidants. The interest in tannins as bioactive components has increased due to their wide range of biological activities, especially pharmacological properties, such as antitoxic, anticancer, antiallergic and anti-inflammatory, anthelmintic, antimicrobial, antiviral, anti-dysenteric etc. (King-Thom Chung et al., 1998; Ghosh, 2015; Sharmaa et al., 2019). Tannins also possess antiseptic properties due to the action of protein coagulation, preventing infection by inhibiting bacterial growth. Naturally, tannins are present in leaves, seeds, bark, roots, fruits and vegetables (Hassanpour et al., 2011; Ghosh, 2015; Sharmaa et al., 2019).
The determination of the tannin content by the titrimetric method showed minimum values characteristic of the cultivar 'Sweet 'n' Sour' in both frozen (1.25%) and dried (1.88%) fruits.

The cultivars 'Amoroso' and 'Fortunella' recorded the same content (1.66%) of tannins in frozen fruits, but not in the case of dried fruits, where the maximum value was achieved by the cultivar 'Fortunella' (2.49%), and the cultivar 'Amoroso' contained about 16.6% less tannins (Figure 3).

Depending on the cultivar, the content of tannins had a maximum difference of 32.9% in dried fruits and 33.4% in frozen fruits. The dependence of tannin content on the method of fruit preservation proved to be more significant, and this difference exceeded 40%.

The research on *Elaeagnus umbellata* fruits carried out in Pakistan (Khattak, 2012) demonstrated a very high content of tannins as compared with our data, namely 126.5 mg/g.

In general, there is very little data on the evaluation of tannin content in autumn olive fruits.

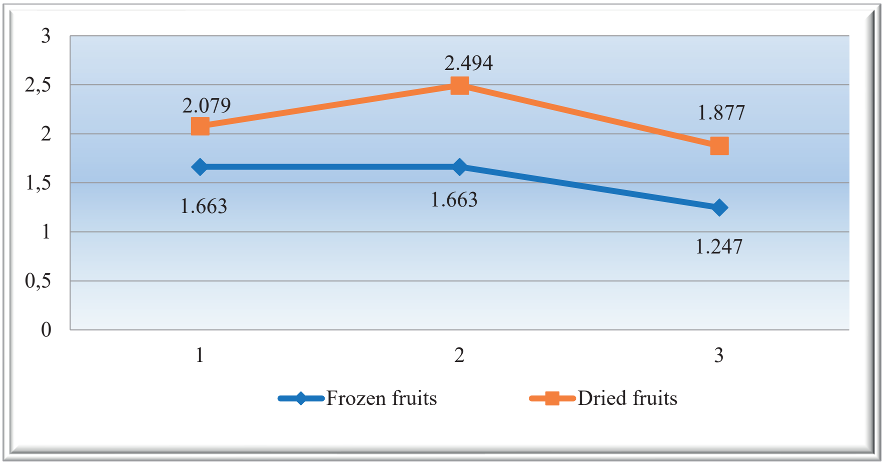

Figure 3. The percentage of tannins in the fruits of different *Elaeagnus umbellata* cultivars (1 - 'Amoroso'; 2 - 'Fortunella'; 3 - 'Sweet 'n' Sour')

Phenolic compounds are secondary plant metabolites and constitute the largest group of phytochemicals, with more than 8000 phenolic structures currently known (Harbone and Williams, 2000), which beneficially influence health due to their bioactive properties. Being important antioxidants, these compounds exert antihyperglycemic, antiviral, anticancer, anti-inflammatory, anti-allergic and antimicrobial activities (Moyer et al., 2002; Manach et al., 2004; Bagchi et al., 2004; Seeram et al., 2006; Badjakov et al., 2008). Berries are rich sources of phenolic compounds, including anthocyanins, phenolic acids, flavonoids, tannins, etc. (Lee et al., 2020). The most common phenolic substances in the human diet are phenolic acids, flavonoids and tannins (King and Young, 1999).

The total content of phenolic compounds, gallic acid equivalent, evaluated in our study ranged from 1037.61 mg GAE/100 g d.m. ('Amoroso' cultivar) up to 1183.64 mg GAE/ 100 g d.m. ('Fortunella' cultivar) in frozen fruits and from 1121.14 mg GAE/100 g d.m. ('Amoroso' cultivar) to 1260.34 mg GAE/100 g d.m. ('Fortunella' cultivar) in dried fruits. Thus, regardless of the type of storage, the fruits of the cultivar 'Fortunella' contain the maximum amount of phenolic compounds, and the cultivar 'Amoroso' - the minimum amount (Figure 4). The fruits of the cultivar 'Sweet 'n' Sour' contain an average amount of phenolic compounds (1176.82 mg GAE/ 100 g d.m. in frozen fruits and 1197.27 GAE/ 100 g d.m. in dried fruits), the value being closer to those of the cultivar 'Fortunella'.

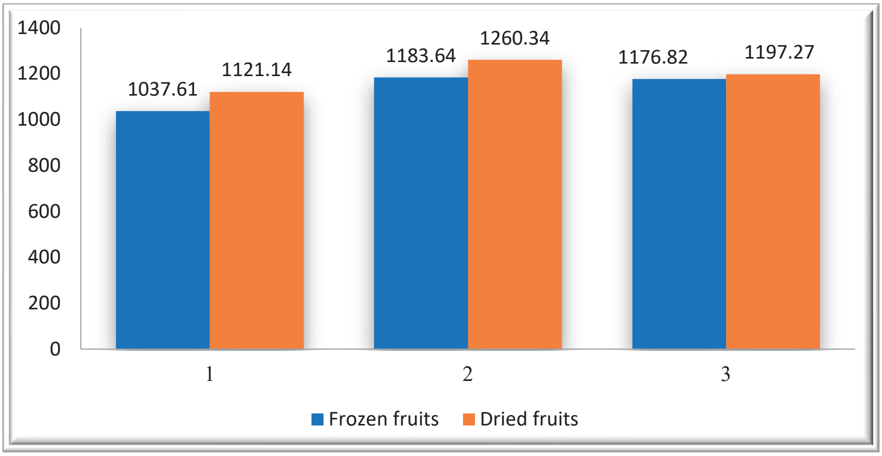

Figure 4. The content of phenolic compounds (mg GAE/100 g d.m.) in frozen and dried fruits of various cultivars of *Elaeagnus umbellata* (1 - 'Amoroso'; 2 - 'Fortunella'; 3 - 'Sweet ˈnˈ Sour')

Similarly, to the other investigated parameters, the amount of phenolic compounds in dried fruits exceeds the respective values in frozen fruits, however this difference is not so great and constitutes only 5.3%. Instead, the biosynthesis and accumulation capacity of these organic substances depends to a greater extent on the genotype, the difference between cultivars reaching a maximum of 14.07% in frozen fruits and a maximum of 12.42% - in dried fruits.

In a study conducted by Surmanidze et al. (2021) on the content of phenols in fruits collected from *Elaeagnus umbellata* plants in different regions of Georgia, it was mentioned that the amount of these compounds in fruits varied greatly, being within the limits of 117.98 mg/100 g and 989.42 mg/100 g, thus demonstrating a very high dependence of the content of phenolic substances on the geographical area. The respective authors also concluded that the impact of climatic factors on this biochemical parameter is very significant. Even lower results have been reported in other papers (Perkins-Veazie et al., 2005; Wang and Fordham, 2007; Gamba et al., 2020). Significantly higher data, however, were presented in an investigation conducted in Pakistan (Khattak, 2012). Thus, a value more than twice higher as compared with the data obtained by us was identified in the fruits of *Elaeagnus umbellata*, namely 2332 mg GAE/100 g. A recent investigation, carried out by a group of researchers in Poland, revealed values of the total phenolic content equal to 1749 mg/100 g d.m. (Zglińska et al., 2021).

CONCLUSIONS

The quantitative determination of ascorbic acid showed relatively close values among the cultivars of the species *Elaeagnus umbellata*, but very different depending on the way the fruits were stored, this difference being about 40%.

The quantification of tannins showed significantly higher amounts in dried fruits in all the investigated cultivars, and in the case of dried fruits, the intraspecific dependence is also very high.

The spectrophotometric determination of phenolic compounds revealed a minimum content in the fruits of the cultivar 'Amoroso' and a maximum in those of the cultivar 'Fortunella', regardless of the type of fruit preservation. The comparative analysis of the obtained data allowed us to conclude that autumn olive fruits are a promising source of phytocompounds and natural antioxidants, which provide them with powerful antioxidant properties, and drying proved to be a more effective method of preserving the fruits than freezing, maintaining a higher amount of active principles.

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propagation and cultivation of new species of woody plants by conventional techniques and tissue culture".

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BY-PRODUCTS FROM THE ESSENTIAL OIL INDUSTRY - VALORIZATION AND RECYCLING. PRACTICAL APPLICATION - ADSORPTION OF TEXTILE INDUSTRY DYES

Anton SLAVOV, Mariyana TOPUZOVA, Gergana MAROVSKA

Department of Organic Chemistry and Inorganic Chemistry, Technological Faculty, University of Food Technologies, 26 Maritsa Blvd, 4002, Plovdiv, Bulgaria

Corresponding author email: antons@uni-plovdiv.net

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 bioadsorbents 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 biosorbents 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.

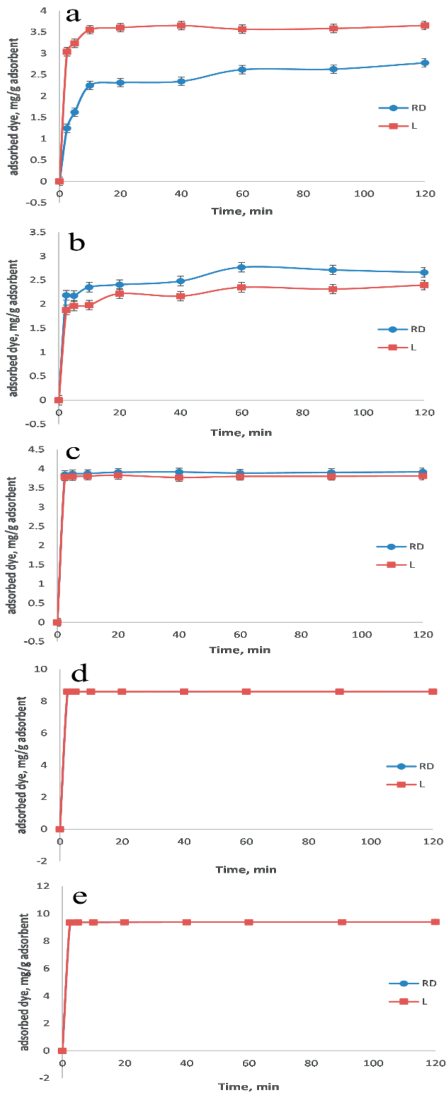

Figure 1. Influence of the contact time on the adsorption of textile dyes (amount of the adsorbent: 1 g; temperature: 20ºC; shaking at 100 rpm); a) initial dye 2; b) spent dye 2; c) spent dye 3; d) spent dye 4; e) spent dye 6

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.

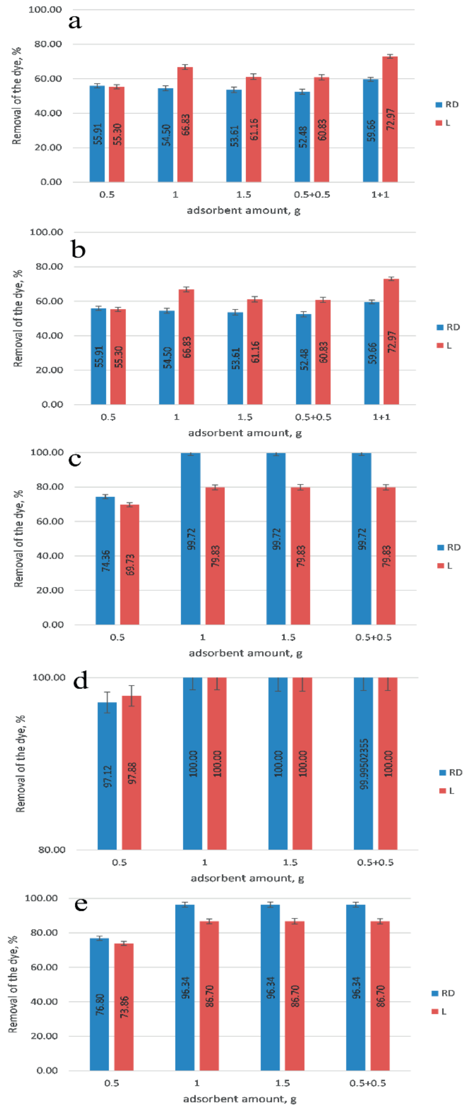

Figure 2. Influence of the amount of the adsorbent/re-adsorption on the adsorption of textile dyes (contact time 20 min; temperature 20ºC; shaking at 100 rpm); a) initial dye 2; b) spent dye 2; c) spent dye 3; d) spent dye 4; e) spent dye 6

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.

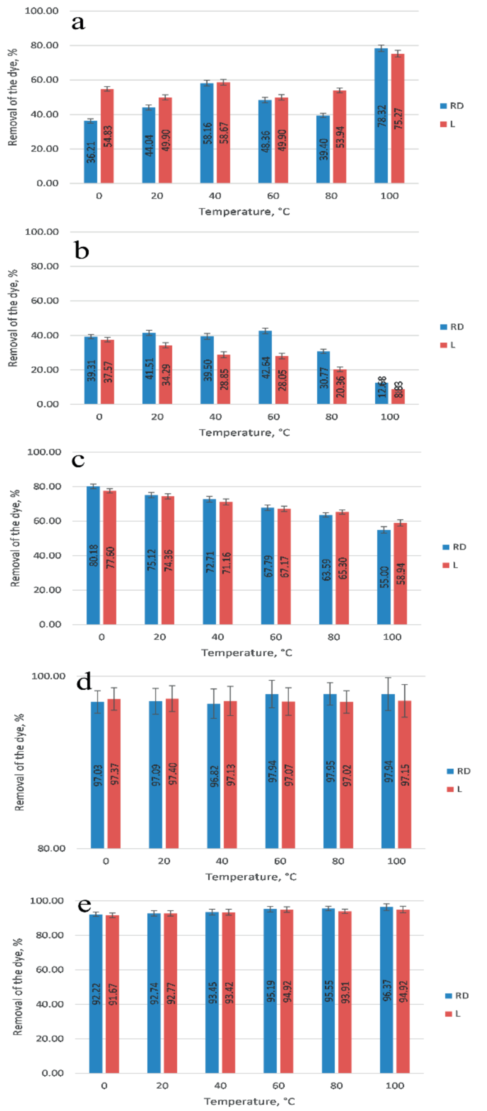

Figure 3. Influence of the temperature on the adsorption of the textile dyes (contact time 20 min; amount of the adsorbent: 1 g; shaking at 100 rpm)

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.

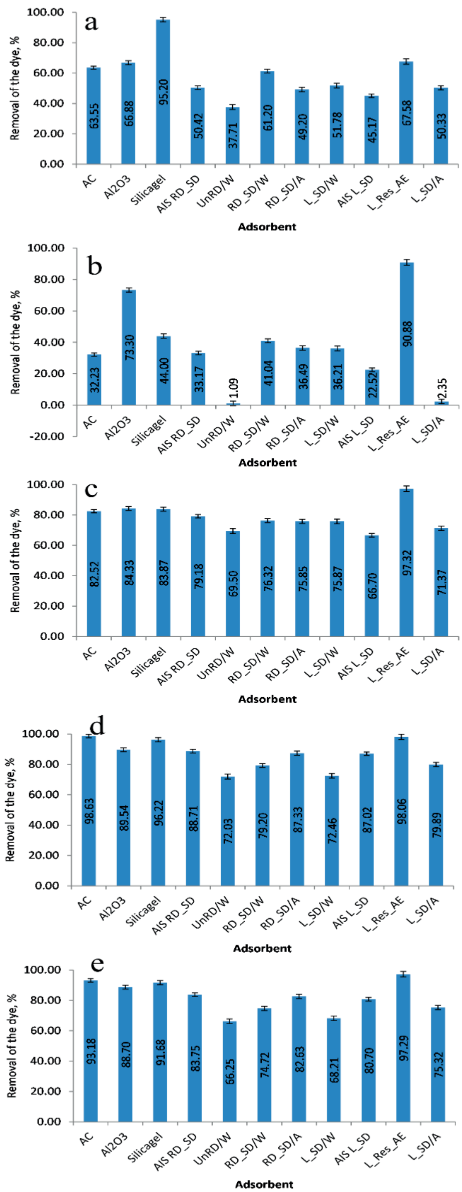

Figure 4. Influence of the type of the adsorbent on the adsorption of textile dyes (contact time 20 min; amount of the adsorbents: 1 g; shaking at 100 rpm). AC - active carbon; Al_2O_3; Silicagel; AIS RD_SD - rose by products washed with 70% ethanol; UnRD/W - dried rose petals (non-distilled) washed 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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RESULTS REGARDING BEHAVIOR OF POTATO CULTIVAR IN MINITUBERIZATION PROCESS DURING 2021-2022

Andreea TICAN, Mihaela CIOLOCA, Monica POPA

National Institute of Research and Development for Potato and Sugar Beet
Brasov, 2 Fundaturii Street, Romania

Corresponding author email: tican_andreea@yahoo.com

Abstract

In order to investigate the effect of variety, nutrion space on minitubers production, a study was carried out in the Laboratory of Vegetable Tissue Cultures of NIRDPSB Brașov, in period 2021-2022. The trifactorial experience (3 x 2 x 2), on 3 repetitions, included the following factors: experimental factor A: cultivar, with three gradations: a1 - Marvis; a2 - Castrum; a3 - Ervant (considered control); experimental factor B: year of study, with two graduations: b1 - 2021 (considered control); b2 - 2022; the experimental factor C: the volume of the nutrition space, with two graduations: c1 - 1.5 l (considered control); c2 - 2 l. In this study Ervant cultivar stands out with a high number of minitubers (7.81), and the Castrum variety obtained the highest value of minitubers weight (75.65 g). The use of increased space for culture has a positive influence on minituberization, both in the number and weight of minitubers obtained/plant.

Key words: *cultivar, minitubers, nutrition space, plantlets, potato.*

INTRODUCTION

Potato is conventionally propagated vegetatively through tubers. The multiplication rate is usually one to ten in one growing season Therefore, it takes several years to multiply a new variety and meet the demand of the growers. Besides, the pathogen population keeps on accumulating generation after generation which lead to the varietal decline. Micropropagation ensures, true to type, disease free and rapid, year-round multiplication of plants. The technique is being used to bulk up new varieties or breeding lines, it is also an ideal material for national and international exchange of germplasm (Kaur et al., 2000).

Protected multiplication using non-conventional approaches, through micro-propagation of nodal cuttings and micro-tuber production followed by further multiplication of minitubers in net houses can be followed to produce high quality potato seed within the region (Ramani and Srivastava, 2010).

In vitro propagated plantlets are commonly used in potato seed production as a source of healthy propagation material (Sharma et al., 2014). Nowadays seed potato (*Solanum tuberosum* L.) programmes worldwide include production of small tubers called minitubers that are grown from *in vitro* derived potato plantlets (Struik, 2007).

Producing minitubers from *in vitro* plantlets allows a faster multiplication rate in seed tuber production programs and reduces the number of field generations needed (Imma and Mingo-Castel, 2006). Prenuclear minitubers of potato (*Solanum tuberosum* L.) are the source material used to produce field-grown seed potatoes (Brendan et al, 1995).

Minitubers can be produced throughout the year and are principally used for the production of pre-basic or basic seed by direct field planting (Lommen, 1999; Ritter et al., 2001)

In vitro produced disease-free potato clones combined with conventional multiplication methods have become an integral part of seed production in many countries (Naik et al., 2000).

Production of healthy potato clones combined with *in vitro* procedures have become an important part of potato seed production, resulting in high quality seed tubers (Jones 1994).

MATERIALS AND METHODS

During 2021 and 2022 years, the experiments were placed in the isolated space of NIRDPSB

Brasov, Laboratory of Vegetal Tissue Culture. The statistical analysis was carried out to establish the influence of the genotype, the study year and the influence of the volume of the culture vessels in obtaining the number of minitubers/plant and their weight. The trifactorial experience (3 x 2 x 2), on 3 repetitions, included the following factors: experimental factor A: variety, with three gradations: a1 - Marvis; a2 - Castrum; a3 - Ervant (considered control); experimental factor B: year of study, with two graduations: b1 - 2021 (considered control); b2 - 2022; the experimental factor C: the volume of the nutrition space, with two graduations: c1 - 1.5 l (considered control); c2 - 2 l. The objective of the conducted research consisted in determining the capacity of plantlets obtained *in vitro* to produce minitubers. The aim was to obtain minitubers from plantlets, after the initiation of meristem culture and the regeneration of healthy plantlets from them, free of diseases, material that will be the starting point in the production of minitubers (obtained *in vivo*).

The experiments for obtaining minitubers were set up in protected spaces of NIRDPSB Brasov, following the location sketch according to Figure 1, a sketch that was respected every year. This experience included 12 variants.

R_1	a_1				a_2				a_3			
	b_1		b_2		b_1		b_2		b_1		b_2	
	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2
R_2	a_1				a_2				a_3			
	b_1		b_2		b_1		b_2		b_1		b_2	
	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2
R_3	a_1				a_2				a_3			
	b_1		b_2		b_1		b_2		b_1		b_2	
	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2

Figure 1. The location sketch of the experimental variants made for three varieties of potato, two years of study and two variants of nutrition spaces (a - cultivar; b - year of study; c - the variety; r - repetition)

RESULTS AND DISCUSSIONS

Regarding variety influence (Table 1) on the average number of minitubers obtained/plant, distinctly significant negative differences are observed for the Marvis (-2.77 minitubers) and Castrum varieties (-1.75 minitubers), compared to the control genotype.

When analysing the influence of the variety on the weight of the minitubers, very significant positive differences are highlighted for Castrum (51.31 g) and Marvis (40.79 g) cultivars, compared to the control variety. For the two years of the study, the control genotype showed a high capacity to produce minitubers, but their weight was low.

Table 1. The influence of cultivar on mean number of minitubers obtained/plant and on mean weight (g) of minitubers/plant for the years 2021-2022

Cultivar (a)	Number of minitub./pl.	Diff./ Sign.	Weight of minitub./pl. (g)	Diff./ Sign. (g)
Marvis (a_1)	5.04	-2.77 oo	65.31	40.79 ***
Castrum (a_2)	6.06	-1.75 oo	75.65	51.13 ***
Ervant (a_3) (Ct)	7.81	-	24.52	-

LSD 5% = 0.92; 1% = 1.52; 0.1% = 2.85.
LSD 5% = 5.52g; 1% = 9.13 g; 0.1% = 17.09 g.

The analysis of study year influence on minitubers number obtained/plant and on their weight highlights distinctly significant positive difference in 2022 year, influencing the parameters studied in a positive sense (Table 2).

Table 2. Influence of study year on mean number of minitubers obtained/plant and on mean weight of minitubers/plant (g) for years 2021-2022

Year of study (b)	Mean number of minitub./pl.	Diff./ Sign.	Mean weight of minitub./pl. (g)	Diff. (g)/ Sign.
2021 (b_1) (Ct)	5.31	-	41.76	-
2022 (b_2)	7.31	2.00 **	68.55	26.80 **

LSD 5% = 1.11; 1% = 1.68; 0.1% = 2.70.
LSD 5% = 14.26 g; 1%= 21.60 g; 0.1% = 34.69 g.

In the two years of study, when comparing the experimental differences with the limit differences calculated in examination of nutrition space volume influence over mean number of minitubers obtained/plant, the positive effect of the increased nutrition space is observed, resulting a very significant positive difference (2.56 minitubers).

Analysing the results regarding the weight of minitubers/plant in the two years of minitubers obtaining, highlights the beneficial influence of the increased nutrition space, expressed by a very significant positive difference (31.72 g) (Table 3).

In 2022, compared to 2021, the Ervant variety stands out with high values of minitubers number (Table 4), with a very significant positive difference (4.88 minitubers). In 2022, by comparing the differences obtained between the Marvis and Castrum varieties, compared to the control genotype, very significant negative differences are found (-4.50 minitubers and -4.33 minitubers).

Table 3. Influence of nutrition space volume on mean number of minitubers obtained/plant and on minitubers weight /plant (g) for the years 2021-2022

Nutrition space volume (l) (c)	Minitub. number/ pl.	Diff./ Sign.	Weight of minitub./pl. (g)	Diff. (g)/ Sign.
1.5 (c_1) (Ct)	5.03	-	39.30	-
2 (c_2)	7.58	2.56 ***	71.02	31.72 ***

LSD 5% = 0.86; 1% = 1.21; 0.1% = 1.71. LSD 5% = 9.24 g; 1% = 12.97 g; 0.1% = 18.31 g

In the period 2021-2022, the Ervant and Castrum cultivars stand out with high values of the minitubers number obtained for the increased nutrition space, determining positive, very significant differences to be obtained (4.71 g and 3.29 g). The increased nutrition space strongly influenced the formation of minitubers for these varieties (Table 5).

The analyse of combined influence of study year and nutrition space volume over mean number of minitubers obtained/plant shows the beneficial effect of the increased nutrition space, for both study years, with distinctly significant differences (1.83 minitubers/2021) and very significant positive (3.28 minitubers/2022). By planting the biological material obtained *in vitro* in the increased nutrition space, a very significant positive difference (2.72) is obtained for the year 2022, compared to the control year. When comparing the results obtained in the culture vessels with reduced volume, a significant positive difference (1.28) is observed in 2022, compared to the control year (Table 6).

Combined influence of variety and year of study on minitubers weight (g) obtained/plant draws our attention to Marvis and Castrum cultivars, which stand out with significant positive differences (37.20 g and 35.78 g), in 2022 (Table 7).

For period 2021-2022, the analysis of cultivar behavior regarding the weight of minitubers obtained on the two nutrition spaces highlights the Castrum and Ervant varieties that determine the achievement of very significant positive differences (60.55 g) and distinctly significant positive differences (24.84 g) for increased nutrition space. The variety/nutrition space interaction draws our attention to: very significant differences by comparing the varieties Marvis and Castrum (48.33 and 33.28 g), with the control genotype, by using the reduced nutrition space. Also, by comparing the previously mentioned cultivars with the control variety, but when using the increased nutrition space, very significant positive differences are observed (Table 8).

Analysing the combined influence of study year and nutrition space volume on minitubers weight highlights the beneficial effect of increased nutrition space for both study years, with distinctly significant and highly significant positive differences (23.71 and 39.73 g). When it was compared the differences obtained for the two study years, a very significant positive difference is observed for the year 2022, compared to the control year for the increased nutrition space (34.80 g) and a significant positive difference (18.79 g) for the reduced space of nutrition, by reporting the year 2022 compared to the year 2021 (Table 9).

Table 4. Combined influence of cultivar and study year on mean number of minitubers obtained/plant for the years 2021-2022

Variery (a)/ Year of study (b)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
	Minitub. number/pl.	Diff./ Sign.	Minitub. number/pl.	Diff./ Sign.	Minitub. number/pl.	Diff./ Sign.		
2021 (b_1) (Ct)	4.33	-	6.21	-	5.38	-	-1.04 ns	0.83 ns
2022 (b_2)	5.75	1.42 ns	5.92	0.29 ns	10.25	4.88 ***	-4.50 ooo	-4.33 ooo

LSD 5% = 1.93; 1% = 2.92; 0.1% = 4.68. LSD 5% = 1.53; 1% = 2.38; 0.1% = 4.01.

Table 5. Combined influence of cultivar and nutrition space volume over mean number of minitubers obtained/plant for the years 2021-2022

Cultivar (a)/ Nutrition space volume (l) (c)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
	Minitubers number/plant	Diff./ Sign.	Minitubers number/plant	Diff./ Sign.	Minitubers number/plant	Diff./ Sign.		
1.5 (c_1) (Ct)	5.21	-	4.42	-	5.46	-	-0.25 ns	-1.04 ns
2 (c_2)	4.88	-0.33 ns	7.71	3.29 ***	10.17	4.71 ***	-5.29 ooo	-2.46 oo

LSD 5% = 1.50; 1%= 2.10; 0.1% = 2.97. LSD 5% = 1.28; 1%= 1.91; 0.1% = 3.06.

Table 6. Combined influence of the study year and nutrition space volume over mean number of minitubers obtained/plant for the years 2021-2022

Year of study (b) / Nutrition space volume (l) (c)	2021 (b_1)		2022 (b_2)		b_2-b_1/ Sign.
	Minitubers number/ plant	Diff./ Sign.	Minitubers number/ plant	Diff./ Sign.	
1.5 (c_1) (Ct)	4.39	-	5.67	-	1.28 *
2 (c_2)	6.22	1.83 **	8.94	3.28 ***	

LSD 5% = 1.22; 1% = 1.72; 0.1% = 2.42. LSD 5% = 1.19; 1% = 1.75; 0.1% =2.68.

Table 7. Combined influence of cultivar and study year on minitubers weight (g) obtained/plant for 2021-2022

Cultivar (a)/ Year of study (b)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
	Minitubers weight. (g)/pl.	Diff./ Sign.	Minitubers weight. (g)/pl.	Diff./ Sign.	Minitubers weight. (g)/pl.	Diff./ Sign.		
2021 (b_1) (Ct)	46.70	-	57.76	-	20.81	-	25.89 *	36.95 **
2022 (b_2)	83.91	37.20 *	93.53	35.78 *	28.22	7.41 ns	55.69 ***	65.31 ***

LSD 5% = 24.70 g; 1%= 37.40 g; 0.1% = 60.09 g. LSD 5% =17.97 g; 1%= 27.42 g; 0.1% = 44.69 g.

Table 8. Combined influence of cultivar and nutrition space on minitubers weight (g) obtained/plant for 2021-2022

Cultivar (a)/ Nutrition space volume (l) (c)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
	Minitubers weight. (g)/pl.	Diff./ Sign.	Minitubers weight. (g)/pl.	Diff./ Sign.	Minitubers weight. (g)/pl.	Diff./ Sign.		
1.5 (c_1) (Ct)	60.43	-	45.37	-	12.10	-	48.33 ***	33.28 ***
2 (c_2)	70.19	9.76 ns	105.92	60.55 ***	36.94	24.84 **	33.25 ***	68.98 ***

LSD 5% = 16.00 g; 1%= 22.46 g; 0.1% = 31.71 g. LSD 5% = 12.13 g; 1%= 17.51 g; 0.1% = 26.17g.

Table 9. Combined influence of study year and nutrition space volume on minitubers weight (g) obtained/plant for 2021-2022

Year of study (b) / Nutrition space volume (l) (c)	2021 (b_1)		2022 (b_2)		b_2-b_1/ Sign.
	Minitubers weight. (g)/pl.	Diff./ Sign.	Minitubers weight. (g)/pl.	Diff./ Sign.	
1.5 (c_1) (Mt)	29.90	-	48.69	-	18.79 *
2 (c_2)	53.61	23.71 **	88.42	39.73 ***	34.80 ***

LSD 5% = 13.07 g; 1%= 18.34 g; 0.1% = 25.89 g. LSD 5% = 13.92 g; 1%= 20.59 g; 0.1% = 31.86 g

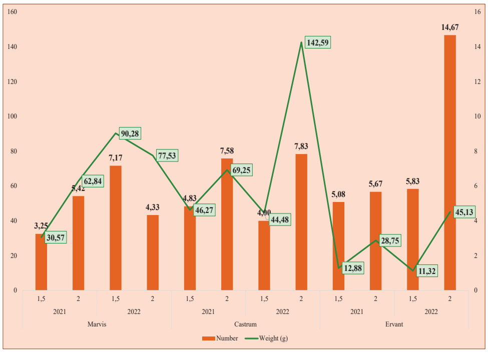

Figure 2. Number and weight of minitubers

Examining the results for two years of study, regarding the number and weight of minitubers/plant, highlights the high capacity of the Castrum variety (Figure 2) to produce minitubers grown in the increased nutrition space for the year 2022 (7.83 minitubers and 142.59 g).

Figures 3 and 4 show the distribution of minitubers number and weight.

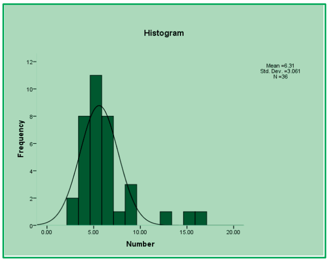

Figure 3. Distribution (Histogram) of minitubers number

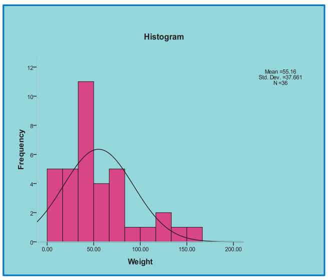

Figure 4. Distribution (Histogram) of minitubers weight (g)

CONCLUSIONS

The Ervant cultivar stands out for recording a high number of minitubers (7.81), and the Castrum variety obtained the highest value of minitubers weight (75.65 g).

The use of increased space for culture has a positive influence on minituberization, both in the number and weight of minitubers obtained/plant.

In 2022, compared to the previous year, higher values were obtained for the analyzed parameters.

In the 2021-2022 period, the Ervant and Castrum varieties stand out with high values of the number of minitubers obtained for the increased nutrition space, causing positive, very significant differences to be obtained. The increased nutrition space strongly influenced the formation of minitubers for these cultivars.

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PRELIMINARY STUDY ON THE INFLUENCE OF THE USE OF VERMICOMPOST AS A CULTURE SUBSTRATE ON THE QUALITY OF LETTUCE SEEDLINGS (*LACTUCA SATIVA* L.)

Laurențiu Liviu URECHESCU, Adrian PETICILĂ, Elena Maria DRĂGHICI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author emails: liviu.urechescu@yahoo.com ; draghiciem@yahoo.com ;

Abstract

The experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Horticulture under laboratory conditions in 2020. During the COVID 19 pandemic, it took place in the university greenhouse, from the end of October until the end of November 2020. The biological material was represented by Red Oak lettuce cultivar, vermicompost (also known as vermicast), peat, zeolite and perlite. The paper aimed to present the benefices of using vermicompost in reducing nutrient solution and a higher yield. We tested 13 variants with 3 repetition each and used vermicompost in different percent as substrate such as 0%, 25%, 50% and 100% of pot volume. The vermicompost was produced by California Red Wigglers from converting two types of precomposted manure, horse and cattle over the period of 26 weeks. The vermicompost has a neutral PH 6,8-7,2 and does not burn the plant. The lettuce from variants where vermicompost was added, had a lower nutrient solution intake, this being an economic effect to reduce water consumption.

Key words: *vermicompost, peat, perlite, zeolite, lettuce.*

INTRODUCTION

Large quantities of animal manure and different organic waste are produced in farming and agricultural production along with sewage biosolids and food waste. If not disposed/treated properly these have the harmful potential of increasing water and soil pollution, globally. With the current world population of 7,999,420,420, we will reach 8 billion people by 15th of November 2022 and 9 billion by 2037 according to https://www.worldometers.info. It's harder and harder to dispose of our garbage in a safe and sustainable way for the environment. A sustainable process for waste and nutrient management is composting and/or vermicomposting. Over 50% of total wastes is organic and can be diverted from the landfills to compost and vermicompost facilities.

Edwards et al., 2011, in "Vermiculture Technology", presents a vermicomposting pilot program started in USA, in different colleges and universities such as The Evergreen State College located in Olympia, Washington, Southern Illinois University Carbondale located in Carbondale, Illinois, University of Oregon located in Eugene, Oregon, University of Massachusetts Lowell located in Lowell, Massachusetts.

Although composting and vermicomposting is happening since the beginning of life on Earth, researchers still don't have an exact definition for compost.

Comforter Compost, Banner Batches and The Complete Compost Gardening Guide use the word "compost" to describe a putrefaction, decay process.

Compost was one of the topics that has interested me since 2008 and over the years I came to the conclusion that you need 5 ingredients to make compost, such as oxygen, water, carbon, nitrogen and time. Adding worms to your compost system, will result in vermicomposting.

Blackburn, 2022, shows that vermicompost is the by-product of vermicomposting or worm composting. Vermicompost typically consists of unprocessed organic materials, microorganisms and worm castings.

Hernández et al., 2010, used vermicompost obtained from cattle manure as a substrate for growing lettuce and they observed that the plants presented a medium and small volume compared to variants cultivated in compost or urea fertilisation.

The substrate is very important for obtaining high quality lettuce seedlings (Draghici et al., 2016).

León et al., 2012, concluded that the volume of lettuce, number of leaves and nitrate content was influenced by applying different proportions of vermicompost to the nutrient solution.

Durak et al., 2017, in his study regarding the effect of vermicompost applied on lettuce, he showed that by applying 200-300 kg/ha of vermicompost, the growth parameters got better, soil quality improved and that lead to an optimal harvest.

Pleasant et al., 2008, mentions that the decomposition of organic materials process is over when you can see it visually and no fermentation odours come from the compost and/or vermicompost system.

Suthar, 2007, used the *Perionyx sansibaricus* (Perrier), a worm for vermicomposting and concluded that the most efficient conversion into vermicompost was from farm waste, sewage and different urban waste.

Tognetti et al., 2013, writes about the quality of vermicompost that it is influenced by the materials used to feed the worms.

Theourn et al., 2022, proves that the increasing of vermicast produced a higher number of active carbon (microorganism carbon energy source) also known as permanganate-oxidizable carbon (POXC).

Payal et al., 2006, did a study and evaluated the efficiency of *Eisenia foetida* (an epigeic worm) on processing organic waste from household, agricultural waste, sewage and fibre from the textile industry and they obtained a valuable vermicompost.

Munroe, 2009, writes about vermicompost's abilities to supress plant diseases and the concept of „soil food web".

Nancarrow et al., 1998, writes about how vermicompost/castings hold their nutrients wrapped around in a membrane. This helps the nutrients to be released slowly so the plant has access and availability as needed to it, over a period of time.

Vermicompost is a natural and organic product, that all farmers should use to improve their soil and production. Mala, 2022, remarks that this organic fertiliser is still unpopular among farmers.

The paper aimed to present the influence of vermicompost on pot substrate, the consumption of nutrient solution, as well as the difference of hight and number of leaves on lettuce.

MATERIALS AND METHODS

The experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture under laboratory conditions, using the metric system.

The biological material was represented by Red Oak lettuce cultivar planted in individual pots for 30 days.

The vermicompost was produced over a period of 6 months from two type of manure, horse and cattle. Prior to feeding the worms, the manure was precomposted for 6 months. Then it was screen and feed to the worms every week, for 26 weeks. After this period, we sifted the worms from the castings (vermicompost) and used it under laboratory conditions for our experiment. The experimental variants consisted in the use of different nutrient substrates of vermicompost (from manure), peat, zeolite and perlite in percent of 0%, 25%, 50%, 75% and 100% also in different proportion (Table 1).

Table 1. The experimental variants

Variant	Substrate type
V1	Peat 100%
V2	Zeolite 100%
V3	Perlite 100%
V4	Vermicompost 100%
V5	Vermicompost 75% + peat 25%
V6	Vermicompost 50% + peat 50%
V7	Vermicompost 25% + peat 75%
V8	Vermicompost 75% + zeolite 25%
V9	Vermicompost 50% + zeolite 50%
V10	Vermicompost 25% + zeolite 75%
V11	Vermicompost 75% + perlite 25%
V12	Vermicompost 50% + perlite 50%
V13	Vermicompost 25% + perlite 75%

The pot experiment started on the 27th of October 2020 and ended on the 27th of November 2020. We used 13 varieties of substrate in different combination with 3 repetition each.
Appearance of lettuce seedlings in figure 1.

Figure 1. Appearance of seedlings

RESULTS AND DISCUSSIONS

Over the course of 30 days, during seedling production, we used a different quantity of nutrient solution, depending on the growing medium. We discovered that for V1 where we used 100% peat for culture substrate, 562.67 ml/plant of nutrient solution was used. With the exception of V3 where we used 100% perlite as a substrate and 615.67 ml/plant nutrient solution. With 53.00 ml more then V1, 9% higher than the control variant (V1). Only V13 variant (vermicompost 25% + perlite 75%) had a significantly similar quantity of nutrient solution added, 562.33 ml.
In the case of using vermicompost at a rate of 100% (V4) the amount of nutrient solution absorbed by the plant was lower compared to the control V1. The amount administered was 466.67 ml with 96.00 ml less nutrient solution than V1, with a distinctly negative significance. We also found that when using vermicompost 75% + peat 25% (V5) the amount of nutrient solution compared to V4 in which only vermicompost was used, was 77.00 ml lower than V1 peat (control).
As the amount of peat in the mixture increased, the amount of nutrient solution used increased, but it was lower than V1-control. In the case of using zeolite in the nutrient mixture in a percentage of 100% (V2), we found that, on average, on the variant, an amount of 463.33 ml of nutrient solution was used, with a percentage of 82.35% less than the control (V1).

A similar aspect was found when vermicompost was used in combination with perlite (Table 2.)

Table 2. The influence of culture substrate on the intake of nutrient solution

VARIANTS	Nutritive Quantity (ml)	Difference (ml)	(%)	Significance
V(0) average	514.41	-48.26	91.42	OOO
V(1)	562.67	0.00	100.00	Ct
V(2)	463.33	-99.33	82.35	OOO
V(3)	615.67	53.00	109.42	***
V(4)	466.67	-96.00	82.94	OOO
V(5)	**485.67**	**-77.00**	**86.32**	**OOO**
V(6)	**551.00**	**-11.67**	**97.93**	**OOO**
V(7)	**553.33**	**-9.33**	**98.34**	**OOO**
V(8)	423.33	-139.33	75.24	OOO
V(9)	522.67	-40.00	92.89	OOO
V(10)	525.33	-37.33	93.36	OOO
V(11)	427.67	-135.00	76.01	OOO
V(12)	527.67	-35.00	93.78	OOO
V(13)	562.33	-0.33	99.94	N

DL 5% = 4.640 DL 5% in % = 0.8246
DL 1% = 6.290 DL 1% in % = 1.1179
DL 0.1% = 8.440 DL 0.1% in % = 1.5000

The appearance of seedlings on experimental variants is shown in Figures 2 and 3.

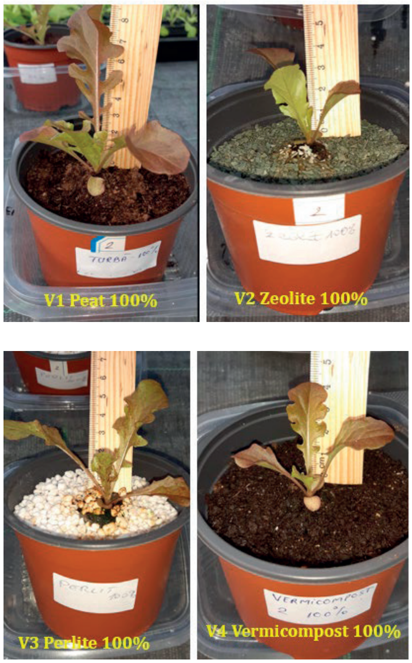

Figure 2. Appearance of seedlings on experimental variants

Figure 3. Appearance of seedlings on experimental variants

Analysing the height of the lettuce seedlings we found that at V1 control - peat 100%, the seedlings had a height of 10.66 cm, compared to the variants in which we used substrate of perlite, zeolite and vermicompost.

The smallest height of 8.67 cm was recorded at V10-Vermicompost 75% + perlite 25%. In the case of variants where vermicompost was used in combination with perlite and zeolite, the height of the seedlings was higher compared to the control variant (Figure 4.).

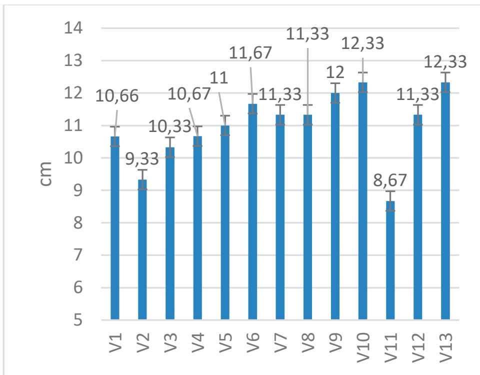

Figure 4. The height of lettuce seedlings after 30 days

It was noted that in the case of V2 in which we used zeolite substrate (V2), the height of the seedlings was 1.33 cm below the V1 - control, aspect noted as well in V3 - perlite, the difference being 0.33 cm, as well as in V11 (Vermicompost 75% + perlite 25%) 1.99 cm.

At the variant where we used 100% vermicompost, it showed a height close to the control version (V1). The highest height of the seedling was identified in V10 (Vermicompost

25% + zeolite 75%) and V13 (Vermicompost 25% + perlite 75%) 1.67 cm above the control V1 (Figure 5).

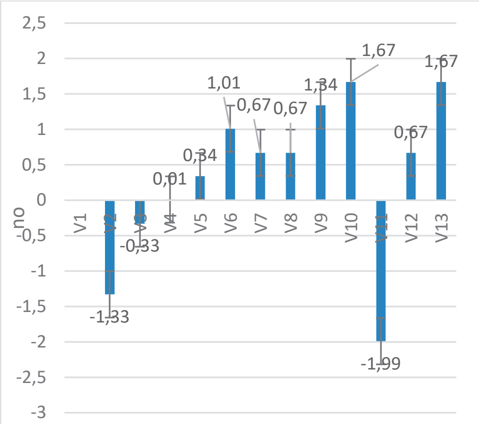

Figure 5. Difference from the control variant (V1 peat substrate) on the height of lettuce seedlings

Regarding the number of leaves we found insignificant differences from the control - V1 between the variants grown on the substrates of zeolite 100% and perlite 100% as well as between V7 - Vermicompost 25% + peat 75%, V9-V13. Significant negative differences were noted in the rest of the variants (Table 3).

Table 3. Numbers of leaves for the lettuce seedlings

Variants	Number of leaves no.)	Difference (no.)	(%)	Significance
V(1)	4.33	0.00	100.00	Ct
V(2)	3.67	-0.67	84.62	N
V(3)	4.00	-0.33	92.31	N
V(4)	3.33	-1.00	76.92	O
V(5)	3.33	-1.00	76.92	O
V(6)	3.33	-1.00	76.92	O
V(7)	4.33	0.00	100.00	N
V(8)	3.33	-1.00	76.92	O
V(9)	3.67	-0.67	84.62	N
V(10)	3.67	-0.67	84.62	N
V(11)	3.67	-0.67	84.62	N
V(12)	4.33	0.00	100.00	N
V(13)	4.67	0.33	107.69	N

DL 5% = 0.910 DL 5% in % = 21.0000
DL 1% = 1.230 DL 1% in % = 28.3846
DL 0.1% =1.660 DL 0.1% in % = 38.3077

CONCLUSIONS

Based on the results obtained, it can be estimated that the largest amount of nutrient solution was used in V3, a variant in which 100% perlite was used.

In the case of using vermicompost, the amount of nutrient solution used throughout the seedling's production was lower, the percentage being 82% nutritional solution compared to the peat-control V1, which means that the substrate retained a higher humidity, a longer time with economic effects to reduce water consumption.

Regarding the height of the seedlings, there were no significant differences, an aspect to be taken into account for the uniformity of the seedling but also for the economic establishment of the components used in the growing substrate.

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INFLUENCE OF MICRONUTRIENT CONTENT OPTIMIZATION ON THE VEGETABLES QUALITY AND YIELD

Maryna ZAKHAROVA[1], Sviatoslav BALIUK[2], Ludmila VOROTYNTSEVA[2]

[1]National Scientific Center «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», 4 Chaikovska Str., Kharkiv, Ukraine
[2]National Scientific Center «Institute for Soil Science and Agrochemistry Research, Chaikovska str., 4, Kharkiv, Ukraine

Corresponding author email: zakharova_maryna@ukr.net

Abstract

Nutritional quality of food is critical role to improving human health. Even mild micronutrient deficiencies in foods have negative health consequences. Finding ways to address micronutrient deficiencies is central to meeting the UN Sustainable Development Goals. The micronutrient content in plant foods depends on their content in the soil. One of the ways to increase the supply of these nutrients (when the content in the soil is low) is to add them when growing the plants. The experiment was carried out in order to investigate the effect of the introduction of micronutrients Zn, Cu and Co in different variants in the cultivation of carrots, beets and cabbage. It was carried out under irrigated conditions. The use of microfertilizers has an ambiguous effect on micronutrient content in vegetables. Zn content slightly increased in cabbage, but almost unchanged in carrots and beets. Cu content increased in cabbage and beets, but almost unchanged in carrots. Co content increased in carrots, beets and cabbage. The use of microfertilizers has increased the yield of vegetables.

Key words*: hidden hunger, irrigation, micronutrient, soils, vegetables.*

INTRODUCTION

The achievement of most of the 2030 Sustainable Development Goals (SDG) (United Nations, 2015) is impossible without eliminating of hidden hunger. The term "hidden hunger" refers to a chronic lack of micronutrients essential to human health, which can't be synthesized by the body, and need to be obtained from the diet. Micronutrient deficiencies compromise immune systems, hinder child growth and development, and affect human potential worldwide (Stevens et al., 2022). Hidden hunger, experienced by about one in three of the world's total population, is a global trend. Micronutrient inadequacies are not only seen in the developing world, but also in the developed world. At the same time, between 720 and 811 million people in the world faced hunger in 2020: they are chronically hungry, lacking calories or protein (FAO, IFAD, UNICEF, WFP and WHO, 2021). That's why achieving food and nutrition security for all is central to the Sustainable Development Goals (SDGs). Ending hunger in all its forms, including chronic and hidden hunger, requires increasing crop production, and improving crop quality and the nutritional value.

Current approaches to address micronutrient deficiencies include various strategies: by medical supplementation and product fortification; by improving the micronutrient content of crop plants; by increasing micronutrient availability in arable soils; enhancing plant nutrient uptake. In the recent past, food fortification using supplements of vitamins and minerals has been a trend in an attempt to provide health benefits for the consumers (Datta & Vitolins, 2016; Lowe, 2021). Improving the micronutrient content of crop plants and increasing micronutrient availability in soils can be achieved through the use of biotechnology, crop breeding, and fertilization strategies, including microbial biofortification (Garg et al., 2018; Buturi et al., 2021; Alam et al., 2022; Denton-Thompson & Sayer, 2022)

Soil is pivotal in food quality and safety: soil composition determines the composition of food (Cogger & Brown, 2016; Dhaliwal et al., 2022). At the same time, soil properties and conventional agriculture with the application of NPK chemical fertilizers, the use of higher yielding crop cultivars, irrigation etc., along with increased yields, can result in an insufficient micronutrient supply to crops (Silver et al., 2021; Dhaliwal et al., 2022). Strategy with application of fertilizers fortified with micronutrients is the simplest and less expensive method among all biofortification methods (Garg et al., 2018; Prasad & Shivay, 2020; Obaid et al., 2022). When crops are grown in soils, where micronutrients become immediately unavailable, not readily translocated to plants tissues to diminish nutritional deficiencies in crop plants targeted application of soluble inorganic fertilizers to the roots or to the leaves are practiced (Ram et al., 2016; Garg et al., 2018; Ishfaq et al., 2022). Improvement of the soil micronutrient status by their application as fertilizers can contribute to decrease in micronutrient deficiency in humans (Garg et al., 2018).

Vegetable crops are generally grown in agro-systems characterized by a high degree of intensification of the production processes and in which the supply of nutrients is increasingly based on the use of fertigation and foliar fertilization, therefore selecting mineral forms and concentrations may have a relevant importance (Buturi et al., 2021).

However, achieving a balanced supply of micronutrients in soils and crops depends on the properties of soil, plants, and, in irrigation, on the quality of irrigation water. In irrigated agriculture, the problem of micronutrient deficiencies is exacerbated, since irrigation waters act as a factor in increasing their migration. Irrigated water with a high micronutrient content can contribute to the accumulation of these elements in soils and plants to dangerous concentrations, which can harm ecosystems and people (Ahmed et al., 2018; Lu et al., 2016; Zakharova et al., 2021).

Therefore, it is necessary to study the effect of the application of micronutrients Zn, Cu and Co in various ways in the cultivation of carrots, beets and cabbage on the yield and quality of plant products under irrigation when there is a deficiency of micronutrients in the soil. Research objectives consisted in evaluation of:

1) mobile forms of Zn, Cu and Co content in irrigated soils and soil quality;
2) Zn, Cu and Co content in irrigation water and water quality;
3) the effect of the application of micronutrients Zn, Cu and Co on the yield of vegetables;
4) Zn, Cu and Co content in vegetables (carrots, beets and cabbage).

MATERIALS AND METHODS

Experimental site

Site is located in the southern part of the Left-Bank Forest-Steppe of Ukraine, Kharkiv district of Kharkiv region. The studies were conducted in long-term stationary field experience with irrigation of Institute of Vegetable and Melons Growing NAAS. Soil is chernozem typical (Chernozems Chernic, WRB). Vegetables (carrots, beets and cabbage) were grown on the site. Irrigation was carried out with using waters Mzha River (national classification of the irrigation water quality). Duration of irrigation is 50 years. Mineralization of irrigation water during irrigation was 0.6-0.8 g/L. Irrigation norms in experiment were (depending on the crops grown and weather condition): 350-1350 m3/ha. Groundwater depth was more than 11 m.

Soil samples collection

Soil samples were selected on an irregular grid with GPS referencing, taking account of soil and lithological heterogeneity. Soil samples were taken on plots from the boreholes. They were collected from the 0-25 cm, 25-50 cm, 50-75 cm and 75-100 cm depth. More than 4 samples of the experiment every culture (carrots, beets and cabbages) were collected immediately prior to harvest with each variant. Irrigation water samples were tested several times during the growing season.

Soil samples analysis

Mobile forms of micronutrients in soils were determined by extraction with ammonium acetate solution at pH 4.8 for one hour using a 1:5 soil: extractant ratio.

Irrigation water and crops analysis

Water samples were analyzed after drying and dissolving the precipitate in 1M HCl. The micronutrients content of crops was determined

by ashing at 550°C for 5 hours and dissolving the ash in 10% HCl.
In all cases, micronutrients were determined by atomic absorption spectroscopy.

Experiment design
The size of each experimental plot was 4 m^2. The experiment was repeated six times, with a systematic stepwise arrangement of 6 variants. Micronutrients were introduced in the form of salts: $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$. Irrigation of the microfield experiment was carried out manually.
The scheme of experiment is presented in Table 1.

Statistical analysis
Statistical processing of the results was performed using Statistica 10 and MapInfo 11.0.

Table 1. Scheme of experiment

Factor	Dose of micronutrients, kg/ha		
	Zn	Cu	Co
Variant 1 - Control, irrigation	-	-	-
Variant 2 - Micronutrients into the soil	3.0	3.0	2.0
Variant 3 - Micronutrients into the soil and foliar nutrition	1.5+1.5	1.5+1.5	1.0+1.0
Variant 4 - Micronutrients foliar nutrition twice during the growing season	1.5+1.5	1.5+1.5	1.0+1.0
Variant 5 - Micronutrients into the soil and fertigation	1.5+1.5	1.5+1.5	1.0+1.0
Variant 6 - Micronutrients fertigation for two irrigations	1.5+1.5	1.5+1.5	1.0+1.0

RESULTS AND DISCUSSIONS

The contents of micronutrients in soil
The average values of the content of mobile form of Zn, Co in the studied soils are slightly lower or at the level of the background soils of Ukraine (Table 2). The average value of the content of mobile Cu in soils is 2.5 times lower than the background, which may be associated with its lower initial content.
The studied soils were characterized by a low supply of plants with Zn mobile forms (Vazhenin, 1976), even if the maximum content of this element in soils is lower than the optimum by 4.2 times. The average Cu content is also insufficient for plants. Plants are provided with Co at an average level.

Table 2. Contents of micronutrients in the 0-30 cm layer of irrigated soils before the experiment, mg/kg

Site		Contents of micronutrients in irrigated soils, mg/kg		
		Zn	Cu	Co
Irrigated soils before the experiment	average	0.99	0.21	0.39
	min/ max	0.72/ 1.18	0.10/ 0.59	0.20/ 0.67
Assessment of soil quality by				
Background[1]		1.00	0.50	0.50
Level of plants micronutrients nutrition[2]	lower	<5.0	<0.5	<0.3
	normal	5.0-10.0	0.5-1.0	0.3-0.7

[1]Authors data
[2]Vazhenin, 1976

Soil contain insufficient amounts of mobile forms of micronutrients necessary for plants, which indicates the need for increasing content of micronutrient in soils. The mobility of micronutrients in the soil, their availability to plants is a very dynamic value, which depends both on internal (chemical properties etc.) and external factors (temperature, acid-base and redox conditions, adsorption properties of the soil etc.) (Kabata-Pendias & Szteke, 2015). Under irrigation, changes in the content of mobile forms of micronutrients in soils, due to more frequent changes in external factors, occur more actively and intensively.
At the same time, Zn, Cu and Co are “potentially toxic elements” and their toxicity manifests itself when the permissible concentrations are exceeded (Kabata-Pendias & Szteke, 2015; Nematollahi, 2020; Raj & Maiti, 2020). It is necessary to study their content in terms of monitoring pollutants and soil, water and crop quality (Baliuk et al., 2017; Malakar et al., 2019; Raj & Maiti, 2020; Zakharova et al., 2021). Therefore, the issues of substantiating the use of micronutrients depending on the levels of micronutrient content in irrigated soils, the provision of them to plants, and determining the most effective ways to use them for specific crops are very important.

The contents of micronutrients in water

The irrigation water used in the experiment is of high quality (Table 3).

Table 3. Content of micronutrients in irrigation water, mg/dm^3 (average ± standard deviation)

Site	Content of micronutrients in irrigation water, mg/dm^3		
	Zn	Cu	Co
river Mzha	0.015 ±0.004	0.004 ±0.001	0.008 ±0.0004
Assessment of water quality by			
Background Forest-steppe of Ukraine[1]	0.016	0.013	0.010
National classification of the irrigation water quality Class1 - Suitable	< 0.5	< 0.08	< 0.02

[1]Authors data

The micronutrients content in water does not exceed the background for the forest-steppe. Irrigation water is of good quality according to the National classification of the irrigation water quality and it is suitable for irrigation. Irrigation water cannot increase the supply of micronutrients to plants and improve their quality.

The impact of micronutrients on vegetable crop yields

Since certain levels of Zn, Co and Cu were quite low in the soil, which could reduce the yields of vegetable crops, and their content in water is low and could not improve the provision of plants with micronutrients during irrigation, we studied the effect of the use of microfertilizers on the quality and yield of crops in a microfield experiment under irrigation conditions.

We explored the impact of the micronutrients use on crop yields (Table 4).

The use of micronutrients for these purposes proved to be effective in all variants and in all crops. Carrot yields increased from 8% (Variant 2) to 47% (Variant 6).

Variants 3, 4 and 5 resulted in yield increases of 41%, 18% and 35%, respectively. Beet yields increased from 11% (Variant 2) to 35% (Variant 3). Variants 4, 5 and 6 resulted in yield increases of 15%, 19% and 23% respectively. Cabbage yields increased from 8% (Variant 2) to 31% (Variant 3). Variants 4, 5 and 6 resulted in yield increases of 10%, 13% and 16%, respectively.

Table 4. The impact of the micronutrients use on crop yields

Factor	Harvest, kg/plot		
	carrot	beet	cabbage
Variant 1 - Control, irrigation	6.8±0.3	10.4±0.4	29.6±0.6
Variant 2 - Zn, Cu, Co into the soil	7.4±0.2,	11.6±0.3	32.0±0.6
Variant 3 - Zn, Cu, Co into the soil and foliar nutrition	9.6±0.2	14.0±0.4	38.8±0.8
Variant 4 - Zn, Cu, Co foliar nutrition twice during the growing season	8.0±0.3	12.0±0.5	32.8±0.7
Variant 5 - Zn, Cu, Co into the soil and fertigation	9.2±0.4	12.4±0.4	33.4±0.7
Variant 6 - Zn, Cu, Co fertigation for two irrigations	10.0±0.4	12.8±0.5	34.4±0.8
LSD_{05}	0.4	0.5	0.9

The most effective, with a significant increase in the yield of carrots, beets and cabbage, was the use of doses of micronutrients twice - fractional application. The best variants were: application of micronutrients with irrigation water for two irrigations; combined use of half doses - into the soil and foliar top dressing; into the soil and application with irrigation water. It draws attention to the fact that the most effective were the options in which the effectiveness of the application of micronutrients (partially or completely in various concentrations) with irrigation water was studied. The least effective, in terms of yield growth, was the variant with the introduction of micronutrients into the soil. Variant using Zn, Cu, Co foliar nutrition twice during the growing season provided an average yield increase.

A large above-ground mass of plants, which is formed during irrigation, causes an increased removal of all nutrients from the soil, therefore, the use of micronutrients in irrigated agriculture and the development of the most effective methods for their application have a significant prospect for development.

The content of micronutrients in tested vegetables

In the conducted microfield experiments, an increase in the content of micronutrients in vegetables was noted (Table 5). The use of microfertilizers had little effect on the Zn content in vegetables. In carrots and beets, an increase in the content of Zn by 1%-3% was noted. The effect in the variant with the use of Zn, Cu, Co foliar nutrition twice during the growing season was better than in the other variants. Cabbage was the most sensitive to the use of Zn microfertilizers in our experiment. In it, the Zn content increased by 10%-15%. The effect in the variant with the use of Zn, Cu, Co fertigation for two irrigations was better than in other variants.

The use of microfertilizers had a greater effect on the Cu content in vegetables. An increase in Cu content by 4%-9% was noted in carrots. The effect in the variants with Zn, Cu, Co foliar nutrition twice during the growing season and Zn, Cu, Co into the soil and fertigation was better than in other variants. In beets, the Cu content increased by 10%-34%. Variants using Zn, Cu, Co into the soil and Zn, Cu, Co foliar nutrition twice during the growing season resulted in an increase in Cu content in beets by 22% and 34%, respectively. An increase in Cu content by 24%-36% was noted in cabbage. The effect in the variants with the use of Zn, Cu, Co foliar nutrition twice during the growing season was better than in the other variants.

Table 5. Content of micronutrients in vegetable (raw vegetables), mg/kg

Factor	Content of micronutrients, mg/kg								
	carrot			beet			cabbage		
	Zn	Cu	Co	Zn	Cu	Co	Zn	Cu	Co
1. Control, irrigation	1.71	0.46	0.07	3.50	0.58	0.12	2.40	0.25	0.08
2. Zn, Cu, Co into the soil	1.75	0.49	0.18	3.54	0.71	0.21	2.65	0.31	0.19
3. Zn, Cu, Co into the soil and foliar nutrition	1.73	0.48	0.17	3.53	0.64	0.28	2.68	0.32	0.19
4. Zn, Cu, Co foliar nutrition twice during the growing season	1.76	0.50	0.18	3.58	0.78	0.28	2.64	0.34	0.20
5. Zn, Cu, Co into the soil and fertigation	1.75	0.50	0.18	3.53	0.68	0.21	2.68	0.32	0.20
6. Zn, Cu, Co fertigation for two irrigations	1.74	0.49	0.22	3.55	0.67	0.23	2.76	0.33	0.19

The use of microfertilizers had the greatest effect on the content of Co in vegetables. The content of Co in carrots increased by 2.43-3.14 times. The maximum increase in the content of this element was noted in the variant with Zn, Cu, Co fertigation for two irrigations. In beets, the content of Co increased by 1.75-2.33 times. The maximum Co content was noted in the following variants: Zn, Cu, Co into the soil and foliar nutrition; Zn, Cu, Co foliar nutrition twice during the growing season. The content of Co in cabbage increased by 2.38-2.5 times. The maximum content of Co was noted in the following variants: Zn, Cu, Co foliar nutrition twice during the growing season; Zn, Cu, Co into the soil and fertigation.

Carrot and beets agronomic biofortification has been carried out with good results – carrot and beets roots have been supplemented with Zn (slightly), Cu (good) and Co (significantly) by application of them as microfertilizers. Cabbage Zn, Cu, Co biofortification has been also carried out with good results after soil agronomic biofortification with an inorganic form of these elements.

The best options providing the greatest increase in micronutrient content in vegetables were the use of half doses of micronutrients: foliar nutrition during the growing season twice; application with irrigation water for two irrigations; application into the soil and application with irrigation water. Attention should be paid to the option with foliar top dressing twice during the growing season. This variant showed an average increase in yield, but it is the leader in the accumulation of micronutrients in vegetables. Application Zn, Cu, Co into the soil (0.5 doses) and foliar nutrition (0.5 doses) was the best option in assessing yield growth, but vegetable quality did not improve significantly.

The experience has shown that it is necessary to constantly monitor the content of micronutrients in soils and irrigation waters, the provision of plants with them. Under the conditions of low and medium supply of plants with micronutrients, the necessity and effectiveness of applying microfertilizers has been proved; with irrigation water. At the same time, it is also necessary to take into account the content of micronutrients in water and their supply with the irrigation rate and adjust the rate of micronutrient fertilizers depending on them. Since different crops have different susceptibility to micronutrient deficiencies, it is necessary to establish and use the most appropriate method of micronutrient application for each crop. The conducted microfield experiment confirmed the possibility of managing the nutritional regime and the possibility of agronomic biofortification of vegetables.

CONCLUSIONS

As a result of the work, the effect of the application of micronutrients Zn, Cu and Co in various ways in the cultivation of carrots, beets and cabbage on the yield and quality of plant products under irrigation when there is a deficiency of micronutrients in the soil was studied.

The average values of the content of mobile form of Zn, Co in the studied soils are slightly lower or at the level of the background soils of Ukraine. Soil contain insufficient amounts of mobile forms of micronutrients necessary for plants, which indicates the need for increasing content of micronutrient in soils.

The micronutrients content in water does not exceed the background for the forest-steppe. Irrigation water is of good quality; it is suitable for irrigation. Irrigation water cannot increase the supply of micronutrients to plants and improve their quality.

The use of micronutrients increased the yield in all variants and in all crops. Carrot yields increased by 8%-47%, beet - by 11%-35%, cabbage - by 8%-31%. The most effective was the use of half doses of micronutrients twice: application of micronutrients with irrigation water for two irrigations; combined use of half doses - into the soil and foliar top dressing; use of half doses - into the soil and application with irrigation water. The variant with the introduction of micronutrients into the soil was the least effective in increasing the yield of all crops. Variant using two foliar nutrition during the growing season provided an average yield increase.

The micronutrient profile of carrot, beets and cabbage has been improved by the application of inorganic microfertilizers. In the conducted microfield experiments, a different increase in the content of micronutrients in vegetables was noted. The use of microfertilizers had little effect on the Zn content in vegetables: in carrots and beets increased by 1%-3%, in cabbage - by 10%-15%. The introduction of microfertilizers had a greater effect on the Cu content in vegetables: in carrots increased by 4% - 9%, in beets - by 10%-34%, in cabbage - by 24%-36%. The use of microfertilizers had the greatest effect on the content of Co in vegetables: in carrots increased by 2.43-3.14 times, in beets - by 1.75-2.33 times, in cabbage - by 2.38-2.5 times.

The best options providing the greatest increase in micronutrient content in vegetables were the use of half doses of Zn, Cu, Co: foliar nutrition during the growing season twice; application with irrigation water for two irrigations; application into the soil and application with irrigation water.

The conducted microfield experiment confirmed the possibility of yeild managment and the possibility of agronomic biofortification of vegetables. Micronutrient biofortification through agronomical practices is an alternative strategy to reduce the Zn, Cu and Co deficiency in carrot, beets and cabbage.

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A REVIEW ON THE ROLE OF MICROBIAL COMMUNITIES IN MAINTAINING PLANT AND SOIL HEALTH

Mihaela ZUGRAVU, Aglaia POPA, Liliana BĂDULESCU, Florentina ISRAEL-ROMING

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: aglaia_popa@yahoo.com

Abstract

Nowadays, the concept of soil health is characterised by three fundamental parameters such as: physical, chemical and biological soil properties. These parameters are in a continuous interaction when are influenced by climatic changes, soil types and the usage of different management practices. The importance of the microbial communities in soil is represented by their ability to decompose the soil organic matter and to transform, mineralize and release essential nutrients important for plant development. In addition, they are important in the detoxification of environmental pollutants and maintenance of soil fertility. In soil, microorganisms are abundant and diverse and include some important taxonomic categories such as: bacteria, fungi, actinomycetes, algae and soil protozoa. This review is focused on the role of beneficial microorganisms in soil health, highlighting the recent advances in this topic.

Key words: *microorganisms, management practices, soil health.*

INTRODUCTION

Soil is an important fraction of the terrestrial environment, which has the quality of supporting all forms of terrestrial life. They maintain and develop life through their property of regenerating, filtering, absorbing, and transforming pollutants.

The agricultural systems sustainability has recently become an important problem all over the globe. Many of this agricultural sustainability problems were connected to soil fertility and the changes that may appear over time. When referring to soil health, it refers to the continued ability of the soil to function as a vital living ecosystem. In addition, soil health is described as well as the unique balance of chemical, physical and biological soil parameters. Soil fertility is an essential fragment of soil quality, that concentrates more on the soil productivity, which is a parameter of the soil's capacity to produce a particular crop under a certain agricultural management system. Most of the productive soils are fertile helping the plant to grow, but some of the fertile soils are unproductive due to the fact they were subjected to harmful natural factors such as drought or management system practices (Nguemezi et al., 2020). These management practices can affect soil nutrients availability through processes such as mineralization, oxidation, leaching and erosion (Liu et al., 2010). Finally, these practices can affect the existence and activities of soil microbial communities and soil structure and fertility (Musbau and Ayinde, 2021).

Modern agriculture has to face new challenges such as climate change, erosion, loss of nutrients from the soil sorption complex, contamination with pollutants and plant protection chemicals, protection of drinking water sources or depletion of soil fertility (Sharma et al., 2017). Furthermore, in modern agriculture, the chemical products for plant protection including insecticides, herbicides and fungicides are used very often to impede the crops from pests and in conclusion to increase the crop production. Once these products enter in the soil, they can perturb soil microbiota and finally the nutritional quality of soil (Kang et al., 2016).

These products for plant protection are applied often throughout the season when crops are cultivated. With every application certain amount of these products infiltrates into the soil. Due to the intensive application of products for crop protection a lot of issues, which include the soil pollution, have appeared. Besides the composition and the quantity of pesticides applied on soil, their impact on the microorganism's activity can depend as well on soil physical, chemical and biochemical parameters (Sethi et al., 2013; Arora and Sahni, 2016).

Soil microbial communities can have a strong impact on soil fertility and crop development, and on the changes in soil structure and dynamics in response to different soil management practices. They can give information about soil quality and biological complexity (Bai et al., 2018; Dusa et al., 2022).

In order to choose the right agricultural management system should be taken into account the microbiological and physio-chemical parameters of the soil. There are various studies that highlighted that most important determinants of soil microbial community structure are environmental factors (temperature, moisture and CO_2 levels), soil physical structure, soil pH and other chemical properties (Schulz et al., 2013; Kiflu et al., 2013; Musbau and Ayinde, 2021). The presence of microorganisms and their variety can be considered as sensitive measure of soil quality. The cultivation systems applied by farmers can lead to changes in the quantitative and qualitative structure of organisms which are living in the soil environment (Kladivko, 2001). In addition, it was highlighted that soil microbial communities are very sensitive to anthropogenic factors, especially agricultural activity (Kuffner, 2004).

SOIL MICROORGANISMS

Soils are a mixture of organic and inorganic matter that host a complex web of organisms that can affect the evolution of soil and various physical and chemical soil parameters. The biological activity was found to be greater in the soil superior part (0-20 cm) and it's diminishing with the soil depth.

Microorganisms are important in all nutrients cycle (N, S, P) from and in the degradation of organic residues. In addition, microorganisms encountered in soil help in the decomposition of the organic matter (OM) derived from plants and animals resulting the energy intake essential for soil functions. After that, the organic matter is transformed to biomass or converted to carbon dioxide, water, nitrogen, and other nutrients (Jacoby et al., 2017).

Microbial populations are sensitive indicators used in the measurement of soil health, a soil quality that cannot be totally defined using physicochemical tools. Microorganisms adapt rapidly to environmental conditions due to their capacity to respond to changes. Therefore, they can be utilized for the assessment of soil health, and the modifications that appear in the activity of the microbial communities (Stott, 2019).

The rhizosphere is a habitat for the development of nitrogen fixing symbiotic bacteria and mycorrhizal fungi, which increase plant performance by improving mineral nutrition. Microorganisms decompose organic matter into plant nutrients which are absorbed by plants. Plants release an increased quantity of different chemical components into the soil. Thus, these chemical compounds lead to the formation of microorganisms structure in the soil. In addition, microorganisms utilize as source of food the root secretions and can produce antibiotics that impede harmful microorganisms. On the other hand, soil microorganisms can release growth regulators and make accessible various nutrients such as P and S that can enhance plant conditions (Furtak and Gajda, 2018). In soil, microorganisms are extremely abundant and diverse and include some important taxonomic categories: algae, bacteria, fungi, yeasts and actinomycetes. From all these categories, two main groups are found more often in the agricultural soils: bacteria and mycorrhizal fungi (Musbau and Ayinde, 2021).

Bacteria lives in soil in various forms. It can be found as cocci which have the form of sphere and the dimensions of 0.5 mm, bacilli with rod form and dimensions between 0.5 and 0.3 mm and spiral. Bacteria is the most predominant category of microorganisms that can be found in soil, their population decreasing with the increase of soil depth (Aislabie and Deslippe, 2013). They are one-celled organisms that constitute the highest biomass of soil organisms. Bacteria are found more abundant near plant roots, one of their main food resources because, in rhizosphere, can be found more abundant the substances secreted by roots, residues from dead plant and animal, sugars, and polysaccharides (Bakshi and Varna, 2011).

Some of the most common bacteria that can be found in soil are part of the genera *Pseudomonas*, *Arthrobacter*, *Clostridium*, *Achromobacter*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Corynebacterium*, *Sarcina*, *Azospirillum*, and *Mycobacteria* (Loper et al. 1985; Bakshi and Varna, 2011).

Soil bacteria can be classified as follows (Ingham, 2009; Aislabie and Deslippe, 2013):

- Symbiotic nitrogen-fixing bacteria that can form connections such as nodules with roots of the leguminous plants. The nodule is the spot where the atmospheric nitrogen is fixed by bacteria and transformed into ammonium which can be assimilated by the plant.
- Nitrifying bacteria which convert NH_4^+ to NO_2^- and then to NO_3^-. Nitrate is leached easier from the soil, so some farmers use nitrification inhibitors to reduce the activity of one type of nitrifying bacteria.
- Denitrifying bacteria, where nitrate is converted into nitrogen (N_2) or nitrous oxide (N_2O) gas. These types of bacteria are anaerobic, meaning that they are active in the absence of oxygen, namely in saturated soils or in the soil aggregates.
- Actinomycetes are a broad group of bacteria that grow in form of hyphae, just like fungi. In soil, actinomycetes are capable to decompose a broad spectrum of substrates, in particular the hard-to-decompose compounds (chitin and cellulose) and are active at increased levels of pH. There are some antibiotics produced by actinomycetes such as *Streptomyces*.

Fungi are microscopic cells. They make their way through soil particles, roots, and rocks with the help of hyphae. They are strongly connected with soil nutrients cycle (C, N, P) due to their capacity to decompose the organic matter (Frąc et al., 2018). Fungi are connected to water dynamics, soil nutrient cycling and biological examination against root pathogens. Furthermore, fungi assist at some processes such as, protection against drought, hormone production, and degradation of plant remaining parts (Treseder and Lennon, 2015; Jayne and Quigley, 2014; Frąc et al., 2018). Fungi have the capability to produce a great diversity of extracellular enzymes. Due to this ability, they are capable to decompose various categories of OM and soil elements and to control carbon and nutrients cycling (Žifčáková et al., 2016). Moreover, fungi can transform the OM into important elements such as biomass, CO_2, and organic acids.

According to the uptake of their energy, fungi can be found in soils under three general functional groups (Ingham, 2009, Bakshi and Varna, 2011):

- Decomposers (saprophytic fungi) are fungi able to transform organic residues into biomass, CO_2 and organic acids. These fungi decompose the complex substrates like cellulose and lignin from wood and are essential in the process of degradation of the carbon ring structures in different pollutants.
- Mutualists (mycorrhizal fungi) are the fungi that colonize plant roots. In rhizosphere, mycorrhizal fungi are able to solubilize phosphorus and transport soil nutrients (P, N and micronutrients) to the plant. They receive in return carbon from the plant (e.g *P. indica*). There are two major categories of mycorrhizae: the *ectomycorrhizae*, and the *endomycorrhizae*. Both of them have a sleeve of external hyphae (approx. 60%) that have the role of absorbing water and ions from the soil, which the fungus gives to the plant through the internal hyphae. The differences between them occurs in the internal hyphae: *ectomycorrhizae* form Hartig's network (substance exchange between the plant and the fungus) only among the cells of the rhizoderm; while in *endomycorrhizae* some of the internal hyphae enter the cortical cells where they differentiate two types of formations: arbuscules (for the exchange of substances between the two symbiotic species) and vesicles (in which the fungus stores reserve substances in the form of glycogen).
- Pathogens or parasites, cause weakened nutrient deficient plant or death when they permeate the plant and breakdown the living tissue. There are various pathogens that increase the economic losses every year. These pathogens are part of genera

Verticillium, *Pythium*, *Phytophthora* and *Rhizoctonia*.

Protozoa are microscopic organisms with only one cell, but larger than bacteria. They are characterised taking into account the ways they move: amoebae use a pseudo (fake) foot, ciliates have cilia (short hair) and move them very fast, and flagellates have one or more flagella (whips) and move them very fast (Tugel and Lewandowski, 2001). Protozoa feed themselves with bacteria, which leads to an increase in bacteria population. In addition, they release a form of N into the soil that can be used by other soil organisms and plants which can't do this by themselves. Protozoa are important in the mineralization of nutrients, making them available plants and other soil organisms use, and help to suppress diseases by competing with or feeding on pathogens.

SOIL MICROBIOME FUNCTIONS THAT SUPPORT PLANT GROWTH

The plant root is an organ which can adapt very well to soil conditions, changing or being changed by surrounding soil parameters (physical, chemical, and biological). The soil area immediately affected by the root with altered microbial diversity, increased enzymatic activity and quantity of organisms, and complex interactions between soil microbial communities and roots is called rhizosphere (Slaughter, 2021). Rhizosphere importance is characterised by the amount of released organic matter by the presence of roots in the soil, followed by an increased number of available nutrients and plant development due to the interaction of microbial communities (Bhattacharyya and Jka, 2012; Helepciuc et al., 2019). The interactions that appear between microbial communities in the rhizosphere are known as the rhizosphere microbiome. In the rhizosphere, the composition of microbial populations, their abundance, and functional attributes are different from the bulk soil microbiome (Mendes et al., 2013).

In the rhizosphere, there are several organisms that have been studied for their beneficial influence on plant development and health: nitrogen-fixing bacteria, arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), biocontrol microorganisms and protozoa (Mendes et al., 2013; Vorholt, 2012). On the other hand, in rhizosphere, can be found some organisms as well that are harmful for plants. This type of organisms includes the pathogenic fungi, oomycetes, bacteria, and nematodes. In addition, another important that can be found in the rhizosphere are the human pathogens (Shah et al., 2021).

For decades, in order to increase crop production was used very often soil bacteria. In soil, bacteria have various functions among which are listed: to make available the nutrients for crops, to stimulate plant development producing plant hormones, to control or inhibit the pathogens that supress plant growth, to ameliorate soil structure, bioaccumulation of inorganics and mineralization of organic pollutants (Shah et al., 2021; Hayat et al., 2010).

Nitrogen fixing bacteria are microorganisms important in the increase of nutrients availability, notably nitrogen. Nitrogen has an essential role in the increase of food and feed production and in the improvement of plant development (Pandey et al., 2020). In addition, nitrogen it is also required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA. Bacteria commonly named as "*Rhizobia*" are considered contributes to the formation of nodosis/bacteriorrhiza/symbioses between nitrogen-fixing bacteria and the roots of leguminous plants. Species of *Rhizobium* (*Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium* and *Sinorhizobium*) have been used globally to allow the nitrogen-fixing symbiosis with leguminous crop plants (Hayat et al., 2010). Over time, the amount of PGPR identified has increased, due to the fact that rhizosphere as an ecosystem has acquired importance in the functioning of the biosphere (Saharan et al., 2011). Rhizobia, which live inside the nodular structures on host roots and assume the form of bacteroides, have a symbiotic relationship with legumes. The rhizobial bacteria transform atmospheric nitrogen into a form that the plant may use in exchange for carbon nutrients from the host, enabling the plant to meet its own nitrogen needs. Nitrogenase enzymes transform atmospheric nitrogen into NH_3 using

ATP as energy source. Biological nitrogen fixation is an essential growth parameter which influences development and yield of the crops. Various species of microorganisms from different genera have capacity of biological nitrogen fixation such as *Bacillus*, *Azospirillum*, *Pseudomonas*, *Klebsiella, Enterobacter*, *Flavobacterium*, *Erwinia*, and *Rhizobium* (Silva et al., 2016). Nowadays, in agriculture is utilised 65% of the nitrogen through the process of biological nitrogen fixation and will continue to be essential for a sustainable crop production (Shah et al., 2021).

Azospirillum colonize the surface and inside of roots. They are known for their capacity to promote plants, stimulating root development and increase the rate of water and mineral intake per root, and maintain soil quality.

Actinomycetes have a critical role in the decomposition of more resistant organic materials such as chitin and in the inhibition of several plant pathogens in the rhizosphere. Furthermore, they are able to decay complex mixtures of polymer in dead plant, animal and fungal material. Through this process can result many extracellular enzymes which are important to crop production (Bhatti et al., 2017). Actinomycetes can be found in the rhizosphere of agricultural crops. In the rhizosphere, they are able to increase soil fertility through organic matter conversion and solubilizing phosphate (Hozzein et al., 2019). Such interactions have made possible to characterize them as plant growth-promoting rhizobacteria (PGPR).

Bacillus is the most abundant genus in the rhizosphere, and the PGPR activity of some of these strains has been known for many years. They are important in the increase of availability of the nutrients that crops need for their development. In addition, *Bacillus* species are known as phosphate-solubilizing bacteria. In rhizosphere, near the roots, *B. subtilis* is capable to maintain stable contact with higher plants and positively influence their growth. *Bacillus subtilis* is also used as a biocontrol agent due to its ability to form endospores and produce different biologically active compounds. In 2004, Garcia et al. have noticed, after the inoculation with *Bacillus licheniformis* on tomato and pepper fields, a considerable colonisation in the rhizosphere. They concluded that this strain can be used as a biofertilizer without affecting normal management in greenhouses (Garcia et al., 2004). *Bacillus* is also found to have potential to increase the yield, growth, and nutrition of raspberry plant under organic growing conditions (Orhan et al., 2006).

Rhizobacteria also play a critical function in increasing soil structure and the growth and stabilization of mineral phosphates.

Arbuscular mycorrhizal fungi (AMF) are plant symbionts associated with the roots of 90% of the plant species, including most important crops, such as cereals, legumes, and members of Solanaceae (Zhu et al., 2010).

Arbuscular mycorrhizal fungi (AMF) are part of *phylum Mucoromycota* and *subphylum Glomeromycotina* (Khaliq et al., 2022). These soil fungi form a complex hyphal network which is productive in mineral and water absorption from a larger surface area. Moreover, the growth of arbuscules occur in cortical cells of roots that empower the fungi with bidirectional resource exchange with the plant (Choi et al., 2018). This type of association can be found at the roots area of about 90% of terrestrial plants, because fungi can supply plants with phosphorous (P) and other mineral nutrients, increase the ability to absorb water and ameliorate leaf photosynthesis (Porcel et al., 2015). This type of fungi leads to several benefits for the plant. Among these benefits are listed: facilitate plant nutrition in providing soil nutrients (phosphorus and nitrogen), protect plant roots against soil pathogen, increases crop yield and mitigate abiotic stresses (Buczkowska and Sałata, 2020; Séry et al., 2016; Floc'h et al., 2022). In rhizosphere, AMF are important in nutrient cycling and in structuring the microbial communities. In addition, the hyphal networks that AMF form in the rhizosphere ameliorate soil parameters such as soil particle aggregation, making the soil more resistant to erosion by wind and water. Finally, AMF decrease nutrient leaching from the soil, having an important contribution in the retention of nutrients in the soil and decreasing the risks of contamination of ground water (Chen et al., 2018). These multiple benefits of AMF make them essential into ecological services in natural circumstances. These fungi are the crucial players of the interactions that appear between soil rhizosphere-Plant-Bacteria-AMF. Furthermore, AMF are used in agriculture as bio-inoculants, and researchers motivate their use as bio-fertilizers in sustainable crop production (Barrow, 2012).

In rhizosphere can be found two major groups of mycorrhizal fungi based on their relational anatomy with host plant roots. The first ones are called septate fungi, which are *Basidiomycota* and *Ascomycota* and are part of the group ectomycorrhizas. Their internal hyphae never penetrate the cells; but they develop among rhizoderm cells and surround the root tips of host plants (Khaliq et al., 2022). The second group contain arbuscular mycorrhizas, ericoid, and orchid, which are considered endomycorrhizas. Their internal hyphae enter and develop in the cells of plant roots (Mbodj et al., 2018).

In various studies, there were highlighted the effect of AMF and PGPR in increasing plant development and protection against pathogens. The process of nitrogen (N) fixation in soil is conducted by *Rhizobia*. Various researchers pointed out that AMF and *Rhizobia* have the same signalling pathway, which stimulates their association with plants (Primieri et al., 2021). In addition, the studies have shown a positive correlation between colonization of plant roots with AMF and the soil microbial diversity (Ferreira et al., 2021).

ABUNDANCE OF MICROBIAL COMMUNITIES IN SOILS WITH DIFFERENT MANAGEMENT SYSTEMS

The agricultural management systems that farmers choose for tillage can influence the physical and chemical parameters of soil and the activity of the microbial communities. Modern agricultural mechanisms, such as the usage of chemical products for plant protection (fertilizers, insecticides, fungicides, and herbicides) allow the defense of crop plants for pathogens and provide better efficiency. These compounds present in conventional agriculture accumulate in soil and can negatively influence the environment and led to soil, atmosphere, and water pollution. On the other hand, organic agricultural management is more environmentally friendly compared to the conventional system. Organic system is characterised by a higher amount of soil organic matter, better soil quality and structure and better protection of the soils against erosion. Moreover, organic managed soils are characterized by a higher biodiversity of plants, animals (pollinators, soil fauna, birds) and soil microbial communities and a greater landscape diversity compared to conventional farms.

In 2008, Birkhofer et al., have noticed that the soils intensively managed frequently contain less fungi biomass. Furthermore, when plant residues are left on the soil surface without being plowed, the quantity of pathogenic fungi to plants may rise (*Fusarium* sp.). Meanwhile, a long-term economy without plowing results in a profound diversification of microbial communities and increases fungal biomass in the soil's top layer. In addition, the number of distinct groups of soil microorganisms is influenced by nitrogen fertilization of the soil. Excessive nitrogen doses used in conventional agriculture, may cause accumulation of toxic compounds, such as ammonia, which is not good for *Actinobacteria* (Natywa et al., 2010).

In response to changes in the composition of organic fertilizers, populations of microorganisms that breakdown cellulose and hemicellulose vary their composition. When organic manure was used as a fertilizer in rice fields, microbial activity increased, but it decreased when chemical fertilizers were used (Mahajan et al., 2016). Moreover, in fields where organic farming was practiced, the populations of *Actinomycetes*, free N-fixing bacteria, and *Azotobacter* spp. increased dramatically compared to conventional system.

In a field experiment involving conventional and organic managements with various tillage intensities (no-tillage, reduced-tillage, and intensive tillage), Hartmann et al. (2015) evaluated soil and winter wheat root-associated microbiomes. Their findings demonstrated that organic farming with intensive tillage for soil and root communities had the maximum diversity of bacteria and fungi. Moreover, the impact of more than 30 years of conventional, no-till, and organic management systems on the soil and soybean-associated microbiomes (roots, stems, and leaves) throughout the plant development stages has also recently been examined by Longley et al. (2020). They added that *Bradyrhizobium* and *Glomeromycotina*, which are known as beneficial organisms for plants, were more prevalent in the roots due to no-till management. Schmidt et al. (2019) demonstrated that there was greater bacterial and fungal diversity in the rhizospheres of organically managed plants compared to conventionally managed plants after using six paired tomato farms in northern California with conventional and organic approaches. Moreover, organic managed farms found to have a higher relative abundance of certain microorganisms that promote plant growth, such as *Pseudomonas*. In a recent thorough study, Ricono et al. (2022) examined the long-term impact of organic and conventional farming on the bacterial and fungal communities linked to the winter wheat roots throughout 40 agricultural areas. According to their findings, compared to conventional farming, the organic one increased the variety of the root microbiomes and increased the abundance of symbiotic fungi (such as *Glomeromycota*) and bacteria that prevent disease, such as *Pseudomonadaceae*, *Burkholderiaceae*, and *Xanthomonadales*, *Gammaproteobacteria*.

Another management practice used in organic agriculture in order to increase the soil organic matter and microbial biomass are the cover crops. Organic fertilizers that replace mineral fertilizers that are known to improve soil organic matter content are typically used in organic agriculture to add significant amounts of C to the soil (Martínez-García et al., 2018). The study conducted by Gattinger et al., in 2012 has demonstrated that (cover) crop residue decomposability variables, such as leaf C: N, greatly influence the rise of soil organic crops stocks in organic versus conventional agriculture. Other studies have shown that the major elements that enhance soil water holding capacity, soil microbial abundance and structure, and weed suppression are cover crop production and its residues management strategies. In a recent study, organic farming has a higher soil organic matter content than conventional farming (Cagnini et al., 2019). The addition of cover crops increased the amount of soil organic carbon and enhanced the organization of the microbial community, soil organic matter, and microbial biomass carbon (Finney et al., 2017). In essence, these alterations are based on the cover crops chemistry and the biotic interactions between plants and soil. Due to rhizobia boosting soil mineralization and the N pool, leguminous cover crops are able to fix an increased amount of atmospheric N (Muhammad et al., 2022).

CONCLUSIONS

For the development and growth of plants, the fertility and health of the soil are crucial. Microbial communities help plants develop more by delivering vital nutrients and minerals that they are unable to use on their own. They breakdown the organic residues so that it can be easily absorbed by plants. In this regard, soil microbes are the primary factor responsible for numerous soil processes influencing the change of nutrients and subsequently affecting the availability of these nutrients to plant roots. The potential of microorganisms to solubilize and mineralize nutrients from inorganic and organic pools is now well understood, and their use could create a new horizon for more profitable and beneficial crop production. One of the major challenges in the current situation is to boost soil productivity by introducing advantageous microorganisms and enzymes without altering the organic structure of the soil. This is because various anthropogenic activities that contribute to environmental concerns have risen.

The presented review indicates that soil is a complex ecosystem, and that cultivation practices have an effect on a variety of soil characteristics (biological, chemical, and physical), as well as the species that live there. Plant growth and consequently yields may be impacted by changes in the populations of soil organisms.

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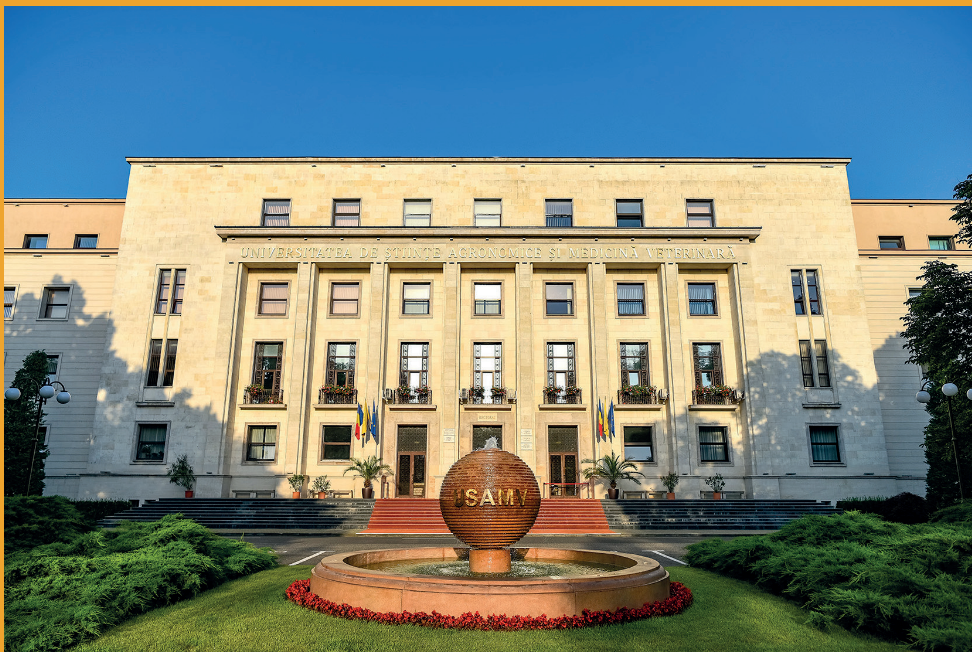

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